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VOLUME 8 NUMBER 8

AUGUST, 1918

Phytopathology

Official Organ of the

American Phytopathological Society

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PUBLISHED MONTHLY FOR THE SOCIETY

OFFICE OF PUBLICATION

2419-21 GREENMOUNT AVE., BALTIMORE, MD.

Entered as second-class matter May 16, 1918, at the post office at Baltimore, Maryland, under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 2, 1918.

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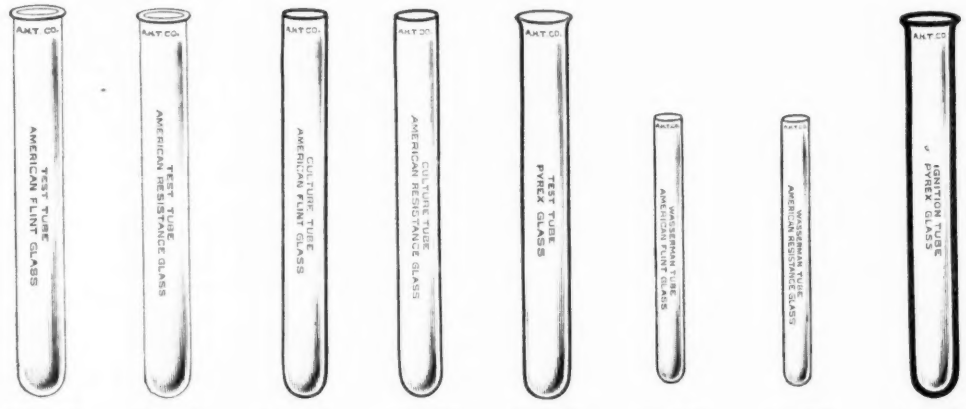

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PHYTOPATHOLOGY

VOLUME VIII NUMBER 8

AUGUST, 1918

THE BLACK-LEG DISEASE OF CABBAGE CAUSED BY PHOMA LINGAM (TODE) DESMAZ.

ERRATA

Due to error the date of issuance of the July number of PHYTOPATHOLOGY was given on page 378 as "July 15, 1918."
The correct date is June 28, 1918.

ceous spots, which elongate, run together and finally come to occupy a considerable area. These areas become paler in color by drying and are then observed to be peppered with "perithecia." The "perithecia" are rounded to slightly oblong, especially on the stem parts, and are provided with an ostiole on the upper surface through which the spore material escapes as a whitish mass. The spores are elliptical in shape, 0.005 mm. in length and each contains two oil droplets.

Prillieux and Delacroix (11) describe a disease of marrow cabbage, which seems to be identical with the black-leg disease of cabbage as it occurs in Wisconsin. The stems are said to be more or less covered with brown-bordered spots which become more and more confluent as the root is ap-

[1] This article is based on work done in the Department of Plant Pathology, University of Wisconsin, 1913–1915, completing investigations opened up in 1912–1913 by R. B. Wilcox. The author is indebted to Mr. Wilcox for advice at the outset, and to Professor L. R. Jones for planning and directing the studies.

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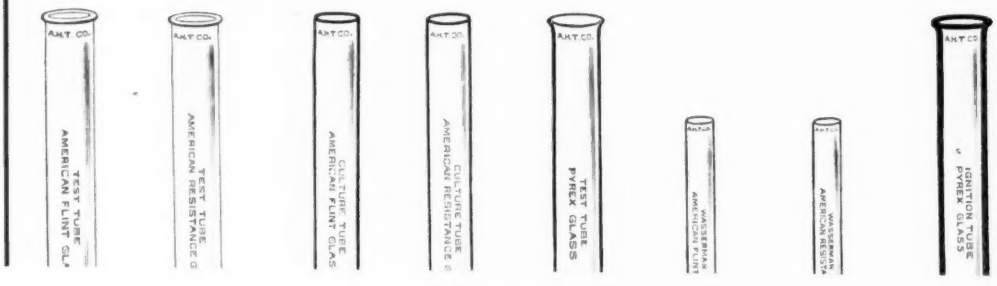

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THE BLACK-LEG DISEASE OF CABBAGE CAUSED BY PHOMA LINGAM (TODE) DESMAZ.

M. P. HENDERSON[1]

WITH TEN FIGURES IN THE TEXT

HISTORICAL

The fungus which causes the black-leg disease of cabbage was first described by Tode (20) in 1791, in his "Fungi Mecklenburgenses Selecti," and was referred to the genus Sphaeria. He found it in Germany occurring on the dried stems of *Brassica oleracea rubra*, but does not speak of it in connection with the living plant.

Phoma in the rôle of parasite on the genus Brassica was first recorded by Desmaziere (4) in 1849 in France. It was found by M. Roberge on the siliques, peduncles and other parts of the panicle of cultivated *Brassica oleracea*, probably the cauliflower. The disease is described in its development on the immature pods as occurring, first, in the form of little olivaceous spots, which elongate, run together and finally come to occupy a considerable area. These areas become paler in color by drying and are then observed to be peppered with "perithecia." The "perithecia" are rounded to slightly oblong, especially on the stem parts, and are provided with an ostiole on the upper surface through which the spore material escapes as a whitish mass. The spores are elliptical in shape, 0.005 mm. in length and each contains two oil droplets.

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proached. The spots are paler at the center and contain numerous, depressed, oval pycnidia whose short necks pierce the epidermis.

Prillieux (12), in a brief note on the foot- or stem-rot of cabbage reiterates the above statements concerning the disease and further states that the pycnidium of the fungus has a rather heavy brown wall, cellular in structure which becomes thinner at the outer end of the neck, and which produces enormous quantities of hyaline spores on its entire inner surfaces.

Delacroix and Maublanc (3) mention the frequent occurrence of the disease in the East of France, especially on the marrow cabbage. The fungus is described as causing large brownish spots covered with little black, slightly flattened "conceptacles." A disorganization of tissues begins at these spots and spreads to the deeper parts of the stem causing the leaves to wither and lose their edible qualities.

McAlpine (9) reports the black-leg or foot-rot as the most serious cauliflower and cabbage disease of Australia. He says the malady is undoubtedly due to a fungus which he recognizes as *Ph. brassicae* Thüm. It was first called to his attention in October, 1897, near Victoria and in the southern part of the country, where losses from it often amount to 25 to 90 per cent of the crop. It thrives best in wet weather followed by heat, the worst attacks occurring when seeds are planted late and the plants are forced by excessive watering.

Under the names of "Fallsucht" and "Krebsstrünke," Ritzema Bos (14) discusses respectively the field and storage phases of a disease as it occurs in the north of Holland, which he attributes to *Ph. oleracea* Sacc. The "Fallsucht" is characterized by the death of the tap-root near the surface of the soil, the soft cortical tissues decaying, leaving only the hard fibro-vascular cylinder. Development of secondary roots just above the dead region is frequently sufficiently strong to maintain the plant, but more often the head becomes too heavy and falls or breaks over. He accounts for the occurrence of the disease in storage by assuming that the fungus enters the young tap-root and remains within the stem in a relatively inactive state, only developing sufficiently to reach the mature head about harvest time. Under storage conditions, discolored areas arise in the stem and from these the infection spreads, first to the nerves, then to other tissues of the leaves, attacking the lower and outer ones first. The invaded tissues are much darkened, and pycnidia arise in great numbers on cut surfaces of stems and just beneath the epidermis of leaf parts. Identity of cause in the two cases was proved by inoculating healthy cabbage heads with pure culture obtained from a plant in the field.

Quanjer (13), also working in Holland, undertook to determine the mode of entrance of the fungus into the cabbage plant under both field and storage conditions. He concluded that the first stages of "Fallsucht"

is identical with the mechanical root injury brought about by *Anthomyia brassicae*, wireworms, and other injurious animals, and that Phoma enters the injured tissues of the already weakened plants. "Other investigations showed that the wilting following transplanting also represents a condition of weakness which makes possible the entrance of the fungus into the injured roots." By pure culture inoculations, he found the fungus to enter readily into the mature head through stomata as well as through the cut surfaces of the stem. "For mature, harvested cabbage heads, the fungus is a direct pathogene, but for seedlings and young vigorously growing individuals, it plays the rôle of a weak parasite." He agrees with Ritzema Bos in his statement that the fungus cannot be carried on the seeds, and that the diseased stumps left in the field are not a source of further infection.

The first authentic report of black-leg in the United States was made by Manns (7, 8) of Ohio. His attention was directed to the disease in the spring of 1910 in Sandusky County, where it was infesting the seed beds prior to the time of transplanting. Further investigation revealed the fact that it already had a rather wide distribution in the cabbage growing sections of the State. "Plants affected with the trouble die in all stages of their growth," but the greatest loss occurs when they are one-half or two-thirds grown. Stems and roots show "sunken lesions extending one-half to two-thirds the distance through the stem," or a collar rot may destroy the stem and allow the top to break off easily.

He regards *Phoma oleracea* Sacc. as the cause of the trouble and considers it "a direct pathogene on seedlings and susceptible plants." Whenever the disease is associated with maggot work, he says, "it simply means that the insect has made it easier for the fungus to get a foothold." He obtained infection by placing washings from diseased plants with seeds at the time of planting, and also observed the rapid spread of the fungus under damp storage conditions.

Harter (6) reports the black-leg as occurring on cabbage in Ohio, Michigan, Iowa, Virginia, Wisconsin, and New York, and on cauliflower in Louisiana. The greatest damage is reported from tide-water Virginia where some fields suffered total loss and in many places 75 per cent of the crop was destroyed. He accepts *Phoma oleracea* Sacc. as the cause of the trouble and says, "infection takes place on the stem near the ground, probably in wounds made by insects, and spreads from that point around the stem, down into the roots and up to the leaves. Leaf infection also occurs at the margins, generally at the ends of the larger veins."

Bailey (2) reports the black-leg as being rather wide-spread in Oregon, where it occurs on cabbage and rape.

STUDIES UPON THE DISEASE IN WISCONSIN

The disease was found in Wisconsin by L. R. Jones, at Onalaska, in the spring of 1911. Here it was evidently of rather long standing, having spread to nearly all fields within a radius of several miles. The damage

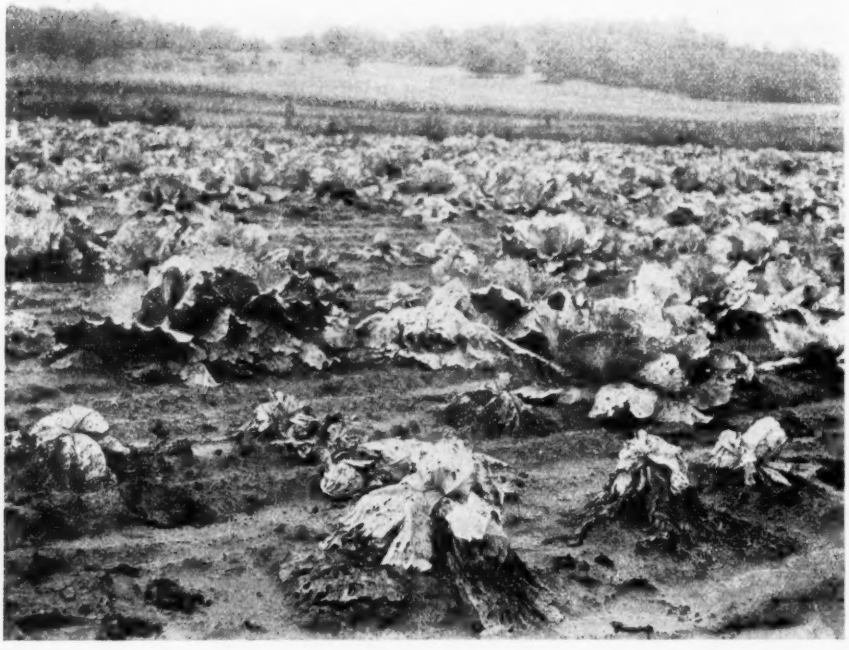

FIG. 1. CABBAGE FIELD DESTROYED BY BLACK LEG

This shows the characteristic wilting and falling over of the plants, of which 75 per cent were already dead or badly wilted. Photograph by L. R. Jones, Onalaska, Wisconsin, August 10, 1911.

varied from the loss of a few heads on some fields to a total failure of crop on others (fig. 1).

In the spring of 1912, R. B. Wilcox (22) took up the study of the disease in the state and by the end of the season had observed it to occur in Dane, Brown, Kenosha, La Crosse, Outagamie, and Racine counties. At

Onalaska, Kenosha, and Green Bay, it was especially destructive. The earliest infection was observed on seedlings two to three weeks before transplanting when they were recognized by their smaller size and pale color. Wilcox states that "a smooth, whitish, sunken area" was found on the stem near the surface of the soil which enlarged rapidly, encircling the stem and extending upward often to the leaves and downward to the roots. The lateral and frequently the tap-roots were entirely destroyed. Pycnidia soon appeared as black dots over the sunken area. The disease became conspicuous in the field within a month after transplanting from an infected seed-bed and, "had developed far enough to show unmistakable symptoms, chiefly wilting" (fig. 2). The sick plants frequently occurred consecutively in a single row, indicating that, "a group of diseased seedlings had been planted one after the other." These formed a source of secondary infection for a considerable area immediately surrounding them. Wilcox concludes that seedling infection is independent of insect or other injury, but thinks the fungus probably cannot penetrate the healthy host tissues during the period between the seedling stage and the dormant condition of maturity.

PURPLING OF FOLIAGE NOT A CHARACTERISTIC SYMPTOM

The writer's observations and conclusions, in general, agree with those of other investigators in this country, and need no further attention. One variation, however, that of foliage discoloration—deserves some consideration at this point.

Purpling of foliage leaves of cabbage has been considered a certain distinguishing characteristic of the black-leg disease. Manns (8) says, "Seedlings about to collapse with the disease take on a bluish-red color of the foliage;" also "the symptoms are very characteristic. The foliage of the affected plants takes on at the margins a mottled, metallic, bluish-red color." Harter (6) says, "Before the death of the plant takes place a purplish tint is developed in the foliage which persists until the plant dies." Wilcox (22) states, "A purplish color of affected plants was also prominent and was a certain symptom of the disease even before wilting took place." He observed, however, on a field at Green Bay, that almost mature plants nearly killed by excessive water show symptoms similar to those of black-leg. "The leaves assume a purplish color, the roots are very often rotted away entirely and the plants collapse and topple over."

The writer made a like observation on a water-logged field near Racine in early summer. Purpling of foliage occurred on practically all plants and the root systems were nearly or quite functionless because of sloughing off of the softer tissues. Numerous attempts to isolate Phoma from these

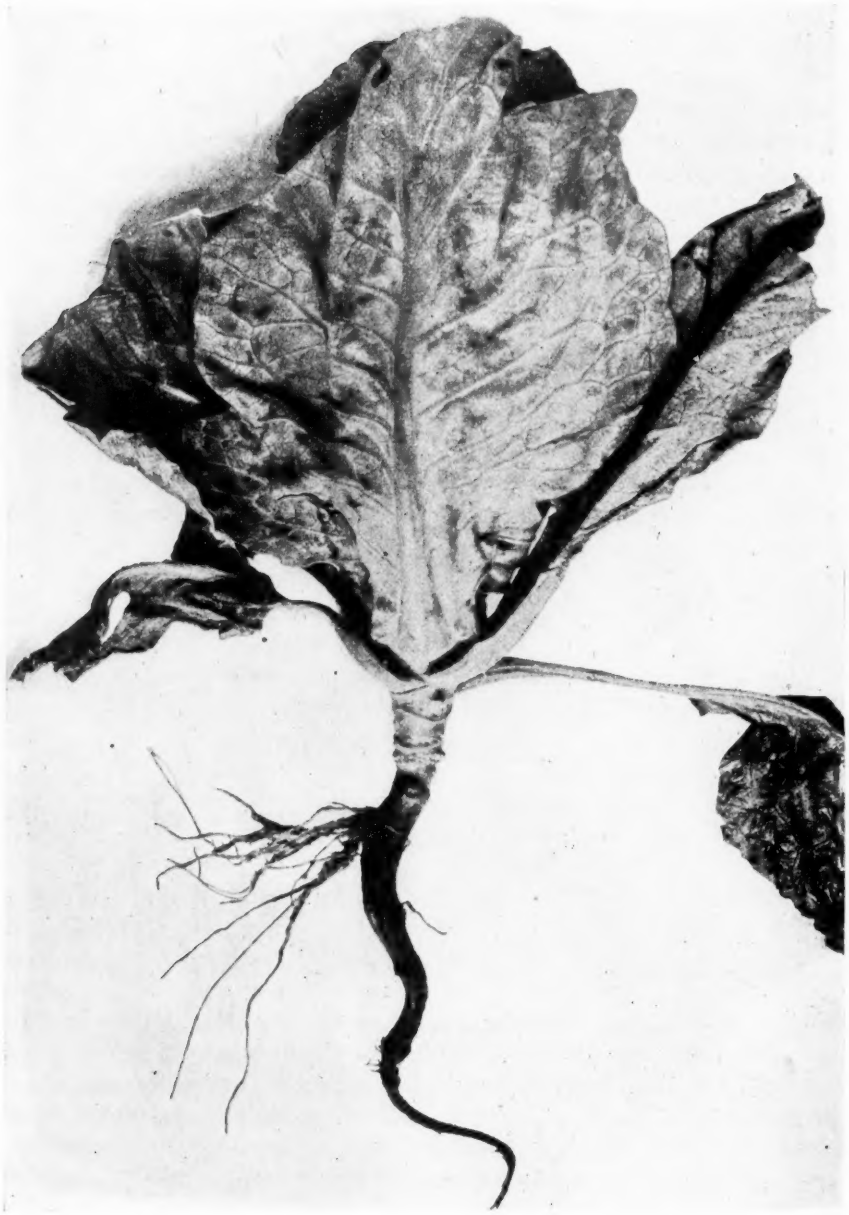

FIG. 2. BLACK LEG OF CABBAGE

Note the death and blackening of the tap root below the soil line leaving only a few weak lateral branches to sustain the partially wilted leaves. Photograph by L. R. Jones, July 10.

plants were without results, and later observations on the field, failed to discover the fungus. At Green Bay, purpling was found to be abundantly developed in plants whose root systems had been nearly or quite destroyed by the club-root organism. It was frequently observed that purpling, whether in connection with Phoma infected plants or not, was not general throughout the leaves of the plant, but occurred rather on that surface or portion of the leaf exposed to the direct rays of the sun. Two factors, at least, appear to bring about this phenomenon: (1) A lessening of the water supply to the leaves by root injury either through Phoma action or in some other way, and (2) exposure to the direct rays of the summer sun. It seems probable that the purple coloring may result from some decomposition product of chlorophyll due to the action of direct sunlight under these unfavorable conditions.

It has been further observed that purpling of the foliage is not general with Phoma infected plants. In the writer's experiments, this phenomenon has been the exception rather than the rule. Of the hundreds of plants which have been artificially inoculated in the greenhouse and have finally died from the effects of the disease, only one showed any purpling whatever, and in that case it could hardly be attributed directly to Phoma action, since all the other plants in the same flat were equally badly infected and many had already been killed. Likewise seed-beds have been destroyed by the fungus, and although they were carefully watched through the entire season, it was not possible to find purpling of leaves on as many as one per cent of the plants. In the field, where the plants were more exposed, purpling was found to be more abundant, but even there it failed to appear on many plants which had been killed by the disease.

From the preceding observations, it is evident that the purpling of the foliage leaves of cabbage, although frequently found associated with the black-leg disease does not necessarily result from it, and cannot, therefore be relied upon as a diagnostic symptom of this disease in Wisconsin.

FUNGUS CAUSING THE DISEASE

Pycnidia

Two types of pycnidia have been found associated with the tissues of cabbage plants attacked by the black-leg disease. Pycnidia of Type No. 1 develop upon the living host tissues where they usually are produced subepidermally, but are also found on other exposed portions where the epidermis has been removed or broken. In these cases the pycnidia are generally associated with definite lesions, depressed and lighter-colored in the interior and surrounded by a distinct purplish border, and they may be either

sparsely distributed or closely compacted, according to the conditions under which they have developed. This type develops abundantly on the soft dormant tissues in storage where the pycnidia may be formed beneath the epidermis or superficially on cut surfaces. It has also been found almost completely covering the surfaces of stems of plants which had been allowed to stand in the seed-bed until mid-summer and then had had their tops cut off three or four inches above the surface. In these cases the weakening of the plants from cutting off of their food supply seemed to permit a more rapid development of the fungus which passed quickly through all of the cortical tissues and produced sub-epidermal pycnidia wherever it reached the surface. No lesions were formed and the epidermis did not appear unlike that of the uninfected plant, except that it was slightly lighter in color and was dotted by the projecting upper surfaces of the pycnidia. Pycnidia of this type have relatively thin pseudoparenchymatous walls and vary in diameter from about 100 to 300 microns. The myriads of spores are discharged through a distinct ostiole at the upper surface in a snake-like stream, which has been found to be either pinkish or whitish in color. This discharge takes place whenever sufficient moisture is applied to the surface, and it seems that spores are continuously produced within, so long as the substratum furnishes sufficient nutriment for the fungus. However, this matter has not been definitely determined.

Pycnidia of Type No. 2 develop on dead tissues within the crevasses and on the surfaces of the woody parts of the host in the early spring. They are somewhat larger than Type No. 1, varying from 160 to 400 microns in diameter, and two-thirds to three-fifths as high when not collapsed; the walls, although approximately of the same thickness, are more compact and resistant and of a dense black color. They are usually provided with a short beak at the middle of the upper surface through which spore discharge takes place. The upper surfaces may be convex, slightly flattened, concave, or variously wrinkled, due to the crowded conditions under which they develop, or to subsequent collapse. The spore mass is whitish in color. The spores have been found to vary from 3.5 to 4.9 microns long by 1.5 to 2.4 microns thick. This type has been demonstrated to belong to the black-leg organism by repeated isolations followed by inoculations of the living host from pure culture. This is undoubtedly the type of pycnidia which Tode (20) had under observation when he wrote the description of *Sphaeria lingam* in 1791.

Mycelium

The mycelium is much branched and, in young culture, sparingly septate and hyaline with numerous refractive droplets; but the wall

becomes yellowish brown with age and septa are very abundant. The threads vary from 2 to 8 microns in diameter. In young infections, the mycelial threads follow the intercellular spaces and pass between the walls of adjacent cells. This is strikingly shown in early seedling infections before the tissues of the hypocotyl have begun to shrivel. The hyphal threads may then be traced for long distances following between two adjacent rows of cells in the direction of the long axis of the stem with only an occasional cross branch. Later the hyphae penetrate the parenchyma cells in all directions, causing a collapse and drying of the invaded tissues; the whole area presents a darkened appearance due to the closer drawing together of the darkened cell walls and the abundance of yellowish to brownish mycelium. Pycnidia develop on tissues of this character, their beginnings being seen as clumps of short, thick, and often very irregular cells containing dense cytoplasm and abundant food material. The xylem elements are also invaded by the mycelium, but this phenomenon is less frequent than the penetration of other tissues.

Cultural characters

Wilcox (22) also tested the cultural characters of three different strains of the organism, one secured from Manns of Ohio and the other two isolated at different times at the Wisconsin Station. They were grown on plain or beef agar, lima bean agar, string bean agar, cabbage decoction agar, oatmeal agar, potato hard agar, rice agar, synthetic culture medium, cooked string bean pods, cooked cabbage plugs, raw sterile cabbage plugs, raw sterile potato plugs, corn meal, and rice. Although "some differences were found in vigor and in tendency to fruit," no constant morphological differences were observed and the conclusion was reached that the strains tested all belong to the same species. "The fungus grows least abundantly upon the synthetic medium and the beef agar" producing scant mycelium and few pycnidia, while, "on all the other media a rather abundant production of both mycelium and fruiting bodies occurs, though in varying degrees." On media, string bean agar for example, with most abundant development of pycnidia, relatively little mycelium was produced; while dense mycelial growth, as on cooked cabbage plugs, was associated with relatively slow and scanty pycnidia formation. "On string bean agar, potato agar, beef agar, lima bean agar, and cooked bean pods, the pycnidia have a reddish or purplish appearance; on cabbage agar, they are almost black, even when first formed; but on the more starchy media, rice agar, rice and corn meal, they are greenish black or almost olive-colored when first formed; on cornmeal, they turn black when about a week old."

NAME OF FUNGUS

The first recognition of this fungus was by Tode (20) in 1791, when he found it occurring on the dried stems of *Brassica oleracea rubra* and gave it the name of *Sphaeria lingam* and described it as follows:

"S. simplex, stylifera, aggregata, subovalis, pulvinata, collabescendo concavorugosa, atra; stylo spermatico breviusculo, decumbente.

"Magnitudine Sphaeriam praecedentem parum antecellit, cui summatim nimium similis invenitur. E. caulibus exsiccatis Brassicae oleraccea rubrae dense pullulat figura ovali, nec non rotunda, pulvinata, superficie laevi, atra. Capsulae, ut primo stylum protrudunt spermaticum, concolorem, nitidulum, jam collabescere hoc pacto incipiunt, ut sulcus plerumque in vertice fungi, dimidio sui cauli herbaceo immersi, extemplo appareat, plus minus hians posthac, in morem labiorum distortorum. Stylus nunquam plenissime obfirmari videtur, certe nutantem fere semper, ac decumbentem inveneris, simulque breviuscnium: quid? Quod haud raro rudimentum ejus solummodo apparet. Sphaeria vieta, si quae alia, multiformis deprehenditur: nunc v. c. complanatam; nunc orbicularem tumida; nunc vero quasi labiatam; nunc demum in labia, seu plicas concentricas, subangulatas, corrugatam cernes."

It is evident from a comparison of this description and accompanying notes of explanation and figures, with the pycnidial forms of the black-leg organism as it occurs with us on the woody tissues of dried overwintered cabbage stems, that Tode (20) was describing specimens of this same fungus. His figures, although crude and diagrammatic, show fairly well the wide variation in size, shape, and wall surface configuration of the pycnidia.

Persoon (10) in 1801 and Fries (5) in 1822 recognized Tode's description of the fungus and the name he had applied to it.

Desmaziere (4), working in France in 1849, found this same species occurring on the dry stems of *Brassica oleracea rubra* and although recognizing it as the fungus described by Tode and designated as *Sphaeria lingam*, he referred it to the genus *Phoma*. His description, which follows, corresponds even more closely with the fungus as it occurs with us. "Le *Phoma Lingam*, assez mal connu, se trouve au printemps sur les tiges desséchées du *Brassica oleracea rubra;* il est peu d'èspeces due genre qui soient aussi polymorphes; ses périthécium varient beaucoup de grosseur; ils sont arrondis, ovales ou difformes, convexes et sans rebords; ou affaissés et même concaves avec un rebord épais. Quelquefois l'ostiole est nulle ou papilliforme; quelquefois elle s'ellonge en petit bec cylindrique et fort caduc. La surface des périthécium est unie, ou marquée d'un sillon figurant un Hysterium, ou bien encore l'on y remarque un ou plusiers plis anguleux ou concentrigues. Au milieu de toutes ces variations, le nucléus

est constamment blanc, solidé, de pourvu de théques, mais contenant de très petites sporidies oblongues, qui renferment aux extrsémité deux sporules globuleuses et opaques; ces sporidies n'ont pas plus de 1–200 de millimètre de longuerr."

During the same year Desmazier (4) described a fungus which he found parasitic on the pods, peduncles, and other parts of the panicle of some species of *Brassicae* and named it *Phoma siliquastrum*. His description of the disease corresponds in all important details with the appearance of *Phoma lingam* in its attack upon mother seed-plants as it occurs in Wisconsin, leaving no doubt whatever as to the identity of the two organisms.

In 1880 Saccardo (17) found a species of Phoma on the stems of *Brassicae oleracea* and gave it the name of *Phoma oleracea* Sacc. with the following description: Perithecia scattered, globular, depressed, papiliolate, 0.25 to 0.35 mm. in diameter, and at first covered. Spores oblong, subcylindrical, sometimes slightly constricted at the middle, obtuse at the ends, biguttulate, hyaline, and measuring 5 to 6 microns long by 2 microns thick.

Ritzema Bos (14), working in Holland on the "Fallsucht" of cabbage, a disease which appears to be identical with our black-leg disease of cabbage, recognized the fungus causing the disease as *Phoma oleracea* Sacc. The pycnidia are described as being at first oval with relatively thick parenchymatous walls. Later they develop nipple-shaped elongations in the middle of the upper surface through which the spores discharge in a rose-colored snake-like mass. They vary from 0.20 to 0.35 mm. in diameter and are two-fifths as high. The spores measure 5 to 5.5 microns long by 2 to 2.2 microns thick and are biguttulate and usually slightly constricted at the middle.

Quanjer (13) agrees with Ritzema Bos in his designation and description of the organism in Holland but the spore measurements which he records are smaller (4.9 microns by 1.6 to 2 microns) and he states that the spores sometimes are biguttulate.

Ritzema Bos (14) and Quanjer (13) recognize *Phoma oleracea* Sacc. and *Phoma brassicae* Thüm. as being identical.

Manns (8) recognized *Phoma oleracea* Sacc. as the organism causing the black-leg of cabbage in Ohio. He says: "The arrangement of the fruiting bodies (pycnidia), their size, 0.25 to 0.35 millimeter in diameter, together with their spore measurements (4.5 to 5μ by 1.7 to 2μ) and other characteristics, all indicate the organism to be the above named fungus."

Wilcox (23) likewise concluded that our black-leg fungus is *Phoma oleracea* Sacc. He says the pycnidia may reach a diameter of 0.30 mm. but many are much smaller. The spores are described as "hyaline, one-celled, cylindrical, rounded at both ends, usually slightly constricted at

the middle, and conspicuously biguttulate; most commonly they are 4.5 to 6 microns long by 1.5 to 2 microns wide," but they are occasionally found 3.5 to 4 microns long.

It is evident from a comparison of the above descriptions that the organism causing "Fallsucht" in Holland is the same as that causing black-leg of cabbage in Ohio and Wisconsin, and that this fungus must have been the one which Saccardo described as *Phoma oleracea*.

The writer has worked with pure cultures of the organisms isolated by Manns in Ohio, Wilcox in Wisconsin, and Bailey in Oregon, and has demonstrated that one fungus is the cause of the black-leg disease of cabbage in these three states. Successful inoculations of this fungus into mother seed-plants have also proved its identity with Desmaziere's (4) *Phoma siliquastrum*. Repeated isolations of the fungus in pure culture from pycnidia of Type No. 2 (described above) and inoculations from these cultures with the production of typical lesions with pycnidia of Type No. 1 have further demonstrated that the fungus is also identical with *Phoma lingam* (Tode) Desmaz.

Numerous pycnidia and spore measurements made by the writer from different hosts inoculated with this fungus, and from different individuals of the same host, show as wide variations as those recorded by different investigators, leaving no reason for doubting the identity of the organisms in this particular.

In 1880, Von Thümen (21), under the name of *Asposphaeria brassicae*, described a fungus which he found on decaying stems of cabbage as follows: "Perithecia large, densely aggregated, sometimes confluent, hemispherical, plicate, superficial, dark brown to black; spores numerous, cylindrical, straight; ends rounded, nonseptate, enucleate, hyaline, 3 to 4 microns long and 1.5 to 2 microns thick. Saccardo later copied this description under the name of *Phoma brassicae* Thüm. and gave Von Thümen credit for the full name, but erroneously so, according to Allescher (1) since Von Thümen did not give a description of *Phoma brassicae* in Hedwigia.

In 1890, Prillieux and Delacroix (11) applied the name of *Phoma brassicae* to a fungus which they found parasitic on the marrow cabbage in France. The stems are said to be covered with spots which are brown at the periphery and lighter toward the center, where they are dotted with numerous depressed oval "perithecia" whose necks pierce the epidermis.

In 1897, Prillieux (12), describing the same disease, still attributes it to *Phoma brassicae* Thüm. and gives figures of sub-epidermal pycnidia.

Delacroix and Maublanc (3) in 1909 used the name *Phoma brassicae* Thüm. to designate the fungus causing the disease of marrow cabbage in France, but also gave the name of *Phoma oleracea* Sacc. as synonymou

with it. The spots caused by it are described as being brownish in color and covered with little, black, slightly flattened "conceptacles." The spores were very small and cylindrical.

McAlpine (9) in 1901 attributes the black-leg or foot-rot of cauliflower in Australia to *Phoma brassicae*, but does not justify his use of the name further than to state, "The disease is undoubtedly produced by the fungus (*Phoma brassicae* Thüm.), and the numerous, often densely crowded perithecia, or spore cases, at the surface, are the fructifications of the fungus."

The above descriptions of *Phoma brassicae* Thüm., although not agreeing accurately in all details, evidently refer to the same fungus. Von Thümen (21) and Saccardo (18) refer to the pycnidia as superficial, while Prillieux and Delacroix (11), Prillieux (12) and Delacroix and Maublanc (3) describe them as sub-epidermal, a difference which might easily result from the conditions under which they developed. Von Thümen and Saccardo describe the fungus as they found it on old decaying stems, while the other descriptions were made directly from the living host.

A comparison of the descriptions of *Phoma oleracea* Sacc. and *Phoma brassicae* Thüm. as recorded by Saccardo reveals three apparent differences between the two. The pycnidia of *Phoma oleracea* are said to be scattered and sub-epidermal while those of *Phoma brassicae* Thüm. are densely aggregated and superficial. The spore measurements for *Phoma oleracea* are given as 5 to 6 microns long by 2 microns thick, while those of *Phoma brassicae* are 3 to 4 microns long by 1.5 to 2 microns thick.

Careful observations on a large number of specimens from different sources make it evident that these differences are not real. The writer has repeatedly found pycnidia of the fungus described as *Phoma oleracea* developing superficially on dried stems and other dormant tissues, where they were also closely aggregated. From his studies and observations, it would be hazardous to state which of the two conditions is more characteristic. On the living host the pycnidia usually develop beneath the epidermis, but are also found on surfaces exposed by injury of some sort, and may be scattered, or closely compacted. On woody tissues exposed to the weather they develop on the surface and are usually densely aggregated, but may also be very much scattered. Under storage conditions pycnidia frequently develop on the surface in a very crowded condition, but here also they may be scattered.

The different spore measurements given for *Phoma oleracea* by the various workers make it certain that this character is also extremely variable and not to be depended upon as a distinguishing specific character. Numerous measurements of spores from different sources have been made with the aid of a Zeiss filar micrometer and their sizes have been found to

vary between such wide limits as to include all measurements previously recorded. The most frequent variation for any single pycnidium, however, was 3.5 to 4.9 microns long by 1.5 to 2.2 microns thick.

The writer has also examined specimens of *Phoma brassicae* sent by McAlpine from Australia and found the pycnidia to be scattered and subepidermal, and the spores to vary from 3.5 to 5.2 microns in length by 1.5 to 2.2 microns in thickness.

From the above considerations, it is clear that the organisms which have hitherto been described as *Phoma oleracea* Sacc. and *Phoma brassicae* Thüm. are identical and that these names are synonyms of *Phoma lingam* (Tode) Desmaz.

In 1892, Rostrup (15, 16) found a species of Phoma on rutabaga roots in Denmark and not being able to identify it with any species already described gave it the name of *Phoma napobrassicae*. The roots were attacked in the field where the action of the fungus was so vigorous that many of them decayed before harvest, but the greater number had a lighter infection confined to the crown region and did not decay until put into storage conditions. The invaded areas were dried and discolored and black pycnidia developed first near the middle of the lesion and later toward the periphery. The spores were 4 to 6 microns long by 1 to 2 microns thick.

It has not been certainly shown that this species is identical with *Phoma lingam* (Tode) Desmaz., but the fact that the rutabaga is so readily attacked by our fungus is very suggestive of that conclusion.

In "Annales Mycologici" for 1913, Saccardo (19) records the occurrence of *Phoma oleracea* Sacc. on the stems of *Melilotus alba* at London, Ontario. The spore measurements are given as 6 to 6.5 microns long by 2 to 2.5 microns thick. The writer obtained samples of this material from John Dearness, who had furnished Saccardo with the original specimens for determination. The fungus was isolated and grown on potato hard agar, on which it fruited abundantly.

Twelve cabbage plants were inoculated from these cultures by placing drops of spore-suspension on the surface of stems, petioles, and leaves, and then wounding the tissues by pricking or slitting them with the point of a sterile scalpel. The plants were then kept in moist chambers at ordinary greenhouse temperature for thirty-six to forty-eight hours. No infection resulted from any of the inoculations. Other plants inoculated with pure cultures of *Phoma lingam* isolated at the same time and in the same manner, and kept under the same conditions as the organism described above showed 100 per cent infections.

Sweet clover plants were inoculated with these same Phoma strains and in the manner described above, except that the plants were growing in

the open so that the moist chambers were provided by covering them with bell jars. Abundant infection resulted from the fungus isolated from *Melilotus alba*, but no infection occurred on plants inoculated with *Phoma lingam*.

Mature pycnidia from the sweet clover tissues were found to vary from 59.2 microns to 240 microns in diameter, the average being smaller than for *Phoma lingam*, and the spores were 4.5 to 7.7 microns long by 2.1 to 3.5 microns thick. The most frequent sizes of spores measured were 6 to 6.8 microns long by 2.4 to 2.8 microns thick. It appears from these experiments and observations that this Phoma on *Melilotus alba* is not identical with *Phoma oleracea* Sacc. and should be referred to some other species.

Unsuccessful efforts were made to secure specimens of the following varieties of *Phoma oleracea* listed by Saccardo (18) in his "Sylloge Fungorum:" *antirrhini, utricae, dipsaci, scrophulariae, solidaginis*, and *helianthituberosi*. It seems possible that a careful test of these might show them to be distinct species of Phoma rather than varieties of *Phoma oleracea*.

GENERAL INOCULATION EXPERIMENTS

A series of preliminary inoculations were made to test the pathogenicity of Phoma, to determine its incubation period after inoculation into the host, and to find whether wounding is necessary to infection.

The first tests were made on four to nine weeks old seedlings. Some were inoculated in the seed bed and not transplanted, others were transplanted and then inoculated, and still others were inoculated at the time of transplanting. The inoculated plants were covered with bell jars from twenty-four to forty-eight hours to prevent too rapid evaporation, and were shielded from direct sunlight by a burlap screen. Four methods of inoculation were used (a) spraying with spore suspension, then pricking the plants at various places with a fine needle on petioles and stems, (b) wounding as above and then spraying with spore suspension, (c) spraying alone, and (d) dipping the roots in spore suspension at the time of transplanting; in this case, the plants being removed from the soil with great care to prevent excessive root injury. Abundant infection resulted in all cases. The disease was noticeable on wounded plants seven to nine days after inoculation, while on others it became apparent about the fourteenth day. Of the plants inoculated by dipping the roots in spore suspension, several died within a week, and others showed characteristic stem lesions with well developed pycnidia at the end of twenty days.

MUSTARD SEEDLING INOCULATIONS

Eight white mustard plants were inoculated by spraying with a spore suspension and pricking the tissues with a needle through the adhering drops. They were kept in a moist chamber for forty-eight hours. Wounds were made on stems, petioles, and leaf-blades. Three weeks later, when the first observations were made, all of the plants were found to be abundantly infected. One was dead. Lesions occurred at all points of wounding, and pycnidia were abundantly developed. The margins of the lesions were purplish, but the color was less intense and not so apparent as in cabbage.

CABBAGE SEED INOCULATIONS

Two pots of steam sterilized soil were planted with about 75 cabbage seeds each. The seeds of pot 1 were thoroughly wet with a spore-suspension of Phoma from pure culture immediately before planting, while those of pot 2, which were to serve as a check, were wet with sterile water.

Ten days after sowing, many of the seedlings in pot 1 were shriveled at the base of the stems and some were already broken over as if "damping off." However, the attacked tissues, instead of presenting a blackened and water-soaked appearance, seemed rather to be withering from a loss of water and were dull grayish-white in color. Closer scrutiny also revealed the fact that the shrunken areas were sparsely dotted with tiny sub-epidermal swellings, the pycnidia slightly yellowish to brownish in color. These became more prominent as they approached maturity. Their color changed to a dark brown, almost black; their further growth, accompanied by the continued shrinking of surrounding tissues, caused them to stand out giving to the thread-like stem an irregular knotted appearance. Many plants showed a slight greenish to purplish discoloration just in advance of the shrunken area as it progressed upward along the stem. The cotyledons had a dull green color when attacked, and were badly shriveled and distorted.

Four plants were apparently attacked in a different manner. The shrinking began with the cotyledons, and, except for moving in the opposite direction, its symptoms were as described above.

At the end of 17 days, when observations were discontinued, only one plant of pot 1 was still standing and it was about one-third as large as those of pot 2, which in every case had remained normal.

The above experiment was repeated, running six inoculated pots in parallel and using one pot as a control. The pots were kept covered with sterile glass dishes for four days at ordinary room temperature after which they were removed to the greenhouse and covered with bell-jars, so ar-

TABLE 1

Cabbage seed inoculations

NUMBER OF POTS	NUMBER OF SEEDS	NUMBER OF SEEDS GERMINATED	PER CENT GERMINATED	CONDITION AFTER 10 DAYS	CONDITION AFTER 12 DAYS	CONDITION AFTER 16 DAYS	CONDITION AFTER 5 WEEKS
1	25	18	72	1 healthy smaller than control. 16 sickly. 1 withered and dead	All infected, dwarfed; 7 dead and withered	7 still alive; 3 with cotyledons infected. Dead plants covered with pycnidia	1 alive but badly infected
2	20	18	90	All smaller than control; 5 infected	5 dead	All dead; Pycnidia on stems and cotyls.	
3	20	17	85	All dwarfed	Still below normal	7 dead and withered	2 standing badly infected
4	20	16	80	5 broken over. 2 dying in upright position	8 dead; pycnidia on stems	14 dead; pycnidia on stems and cotyls.	1 alive; base of stem withered
5	15	12	80	2 withered at base. 11 dwarfed. 1 normal	7 dead; pycnidia on stems. 1 normal	2 alive; cotyledons of 1 withering, 1 normal	2 still alive; 1 withered at top, 1 normal
6	15	12	80	6 withered. All below normal	All dead; pycnidia on stem and cotyledons		
Control	20	17	85	Normal, 2 times size of others	Have gained more over other pots	2 damping off at base	Same as at last observation

ranged as to admit air at the bottom. They were kept moist with distilled water which was at first poured over the surface and later allowed to pass up through the bottom opening.

The results of this experiment as summarized in table 1 point to the following conclusions:

Phoma seed-inoculation, although producing nearly 100 per cent infection has little or no effect on the percentage of germination. The average for the infected pots is less than 4 per cent below that of the control, a difference readily attributable to a variation in viability of the seeds planted. In pot 1, where germination is 13 per cent below the control, it is possible that the slightly more crowded condition may have increased

FIG. 3. INFECTION EXPERIMENT WITH PHOMA LINGAM ON CABBAGE

The two pots at the sides sprayed with spore suspension; control pot in the middle.

the possibilities for early infection so that some of the tender seedlings succumbed to the disease before appearing above the surface. This is also borne out by the fact that infection was slightly more vigorous in this pot on the tenth day than in any of the others. However, in pot 2, with a germination of 90 per cent, five per cent above the control, the attack was so vigorous that the plantlets were all dead at fourteen days; so it appears that difference in germination as indicated by the number of plants which appeared above the surface, is to be accounted for chiefly through the variations in viability of the seeds sown.

It is evident that infection took place very soon after germination and that a relatively long vegetative period of the fungus preceded the actual

destruction of the plant tissues. Prior to the tenth day after planting, the infected seedlings could not be distinguished from the controls, except by their smaller size. This characteristic, however, was so uniform that it must have been a direct result of early infection (fig. 3). This is strikingly illustrated in pot 5, where all of the plants, except one, were dwarfed and badly infected; this one was apparently normal at the end of three weeks when observations were discontinued. This assumption is further borne out by the very rapid collapse of the tissues after the first appearance of the disease on the surface.

In another seed inoculation experiment, the spore-suspension was prepared by carefully removing the pycnidia from an old stump, which had lain in the field over winter, and putting them into sterile water. Two pots of sterile soil, containing thirty-five seeds each were inoculated as above described and a third pot was run as a control. They were kept moist with distilled water. The results of this experiment were identical with those of the two previous trials, except that the action of the fungus on the host was not nearly so vigorous. The first infection was observed on the fourteenth day and pycnidia did not develop until two weeks later.

The preceding experiments indicate (1) that *Phoma lingam* (Tode) Desmaz. is a vigorous pathogene on the cabbage, infection resulting readily from (a) wound inoculations either before or after application of the spore-suspension, (b) spraying without wounding, (c) wetting of roots in spore-suspension at time of transplanting, and (d) wetting of seeds with spore-suspension made either from pure culture or from old pycnidia on over-wintered stumps; (2) that the incubation period of the organism varies from seven to twenty-eight days, pycnidia developing usually in about two weeks; (3) that seed inoculation, although not reducing germination, does result in very early infection of the seedlings as is shown by their much smaller size and slower rate of growth; (4) that the host range of *Phoma lingam* may be much wider in the Cruciferae than has hitherto been suspected.

EXPERIMENTS TO DETERMINE HOST RANGE

The readiness with which Phoma had attacked the white mustard in a previous experiment suggested a wider host range than had hitherto been attributed to it. Accordingly various Cruciferae were inoculated with the fungus to ascertain its possible distribution in this group of plants and the relative susceptibility of the different members of it.

Seedling inoculations

The first experiments were with ten-day-old seedlings. These were inoculated either by spraying with a pure culture spore-suspension in

sterile water, or by spraying and then pricking the epidermis with a fine sterile needle. The plants were kept in a damp chamber for forty-eight hours, and were liberally supplied with tap-water during the whole period of observation.

Rutabaga (*Br. campestris*). Of six rutabaga seedlings inoculated by spraying, two were dead and withered and the stems of two others were badly shrunken on the fourteenth day. Pycnidia had developed on the seed leaves. Four days later, all of them showed abundant pycnidia on stems, hypocotyls, or cotyledons. They were considerably smaller than the check-plants.

Brussels sprouts (*Br. oleracea* var. *gemmifera*). Eight Brussels sprouts seedlings were sprayed with a spore suspension. They developed more slowly and were much smaller than the check-plants. Two weeks after inoculation, they were all badly diseased, and four days later were dead with fully developed pycnidia plentiful on hypocotyls and seed leaves.

Cauliflower (*Br. oleracea* var. *botrytis*). Eleven cauliflower plantlets were inoculated, seven by spraying and four by spraying and wounding. The wounding was evidently unnecessary since infection was as general and as severe on the unwounded as on the wounded plants. On the fourteenth day they were all much withered and developing pycnidia on cotyledons and hypocotyls. The latter showed a very dark purplish color which extended also to the petioles of the seed leaves. The cotyledons had become a much darker green.

White mustard (*Br. alba*). Seven white mustard plants were inoculated by spraying and nine by spraying and wounding. The unwounded seedlings grew much faster than the wounded ones, and, with one exception, were two to three times as large. All of these plants showed cotyledon infection on the fourteenth day, pycnidia developing on some; four days later, the disease had spread to several of the foliage leaves. The nine wounded seedlings grew very slowly after inoculation and, on the fourteenth day, were all badly infected. . Pycnidia were abundant on the cotyledons.

Black mustard (*Br. nigra*). Nine black mustard seedlings were sprayed and twelve were inoculated by spraying and wounding. Of the first lot, two had broken over at the base of the hypocotyls and several lighter colored spots had appeared at the margins of the cotyledons on the fourteenth day. Pycnidia did not later appear on either of these plants, and the apparent infection was not demonstrated to be due to Phoma. Three of the wounded plants were withered at and for a short distance above the surface of the soil two weeks after inoculation. Pycnidia could not be detected at this time but had developed in the shriveled tissues of the hypocotyls at the end of another week. All of the other plants remained healthy and apparently normal as compared with the control specimens.

It appears from the above data (see table 2) that rutabaga, Brussels sprouts, and cauliflower were all readily attacked by *Ph. lingam*, and that they are about equally susceptible to it, at least in the young seedling stage.

TABLE 2

Seedling inoculations

PLANTS USED		HOW INOCULATED	CONDITION 14TH DAY	CONDITION 18TH DAY	FIRST APPEARANCE OF PYCNIDIA	PER CENT INFECTION
Kind	Number					
Rutabaga	8	Spray	2 dead, 2 badly withered. 4 dwarfed	All withered down	14th day	100
Brussels sprouts	8	Spray	5 badly withered. All dwarfed	All withered down	14th day	100
Cauliflower	7	Spray	6 cotyledons withered. 1 hypocotyl withered	All withered down	14th day	100
Cauliflower	4	Spray and wound	3 cotyledons withered; 1 hypocotyl withered	All dead	14th day	100
White mustard	7	Spray	2 normal 5 cotyledons withered	2 normal. 5 infected but erect and vigorous	14th day	71
White mustard	9	Spray and wound	1 hypocotyl withered. 8 cotyledons withered. All dwarfed	All withered down	14th day	100
Black mustard	7	Spray	2 hypocotyls broken at base and cotyledons with light spots. 5 healthy	2 sickly* 5 healthy		5
Black mustard	12	Spray and wound	3 hypocotyls, withered at base. 9 healthy	3 infected in hypocotyls. 9 healthy	21st day	26

* Infection not demonstrated to be due to Phoma.

The white mustard, although readily infected is slightly more resistant to attack, especially in the uninjured condition, and seems to retain its vitality longer than the other species mentioned. The black mustard

appears to possess a high degree of resistance to Phoma. Only 25 per cent of the wound inoculations produced infection, and of those made without wounding, none could be demonstrated to have caused disease of the plant.

Seed inoculations

Other experiments to determine the host range of *Phoma lingam* were carried out by means of seed inoculations. The soil, after potting, was steam-sterilized in the autoclave at 5 pounds pressure for a period of five hours, each pot being covered with an inverted glass dish. The seeds were sown on the sterile soil, thoroughly wet with a suspension of spores from pure culture in sterile distilled water, and then covered with a layer of sterile soil. The pots were kept covered with the sterilized lids for four days. They were then put into a large chamber provided with glass top and sides, so that they might be kept free from insects and where the moisture could be better controlled. The covers were removed and placed under the pots to serve as water receptacles, by means of which distilled water was supplied to the plants through the experiment, this method of watering being employed because it reduced the possibility of splashing infection from the inoculated to the control pots, and seemed the better plan for keeping the surface layers of the soil uniformly moist. Except in a few cases, which are indicated later, one inoculated and one uninoculated pot, each containing 60 to 75 seeds, was used. The controls were treated in every way like the inoculated pots, except that the seeds were wet with sterile water at planting.

The following members of the Cruciferae were thus tested: rutabaga (*Br. campestris*), cauliflower (*Br. oleracea* var. *botrytis*), kohlrabi (*Br. oleracea* var. *caulo-rapa*), kale (*Br. oleraceae* var. *acephala*), rape (*Br. napus*), turnip (*Br. rapa*), Chinese mustard (*Br. juncea*), black mustard (*Br. nigra*), charlock (*Br. arvensis*), radish (*Raphanus sativus*), garden cress (*Lepidium sativum*), peppergrass (*Lepidium virginicum*), stock (*Matthiola incana*), sweet alyssum (*Lobularia maritima*), tumble mustard (*Sisymbrium altissimum*), hedge mustard (*Sisymbrium officinale*) and shepherd's purse (*Capsella bursa-pastoris*).

Of the species and varieties used in these experiments (see table 3), only two, shepherd's purse and peppergrass, failed to show Phoma infection. This may possibly be due to delayed seed germination, other experiments having indicated that the spores do not remain viable for a very long period after escaping from the pycnidia. Sweet alyssum and hedge mustard were moderately susceptible, showing only 10 per cent and 20 per cent infection respectively, and in both cases external evidence of the fungus did not appear until about the seventeenth day after planting.

All others were very susceptible, 50 per cent to 100 per cent of the plants being attacked. Infection was first manifest in the smaller size and darker green color of the diseased seedlings. This symptom was apparent almost from the first appearace of the plantlets above the soil and became intensified as the disease progressed. In most cases the attack of the fungus began at the base of the hypocotyl which usually was shriveled and pycnidia were evident by the twelfth day after planting. Occasionally the attack began at the cotyledons and the shrinking of the tissues extended down the stem.

Another set of seed inoculations similar to the above was carried out during the summer, but with some slight variations. Pots of garden soil were inoculated by mixing a fourteen-day-old pure culture of Phoma on potato hard agar, into the surface layers of each. The seeds were sown on this surface and covered with another layer of soil. Rutabaga, kohlrabi, kale, rape, turnip, Chinese mustard, black mustard, charlock, radish, garden cress, stock, sweet alyssum, tumble mustard, hedge mustard, and shepherd's purse were thus planted. The plants were kept in the open greenhouse and were watered with tap-water at regular intervals, no attempt being made to control the humidity of the atmosphere about them or to keep them free from insects. Observations were made at the end of three weeks after planting, at which time all of the pots except shepherd's purse showed infection. The diseased seedlings were perceptibly smaller than the controls. The plants were not examined individually and counted to determine the percentage of infection, but their relative susceptibility was shown to be approximately as given in table 3, except that the radish was much more resistant than is there indicated.

Two pots of steam-sterilized soil were planted with each of the following crucifers: collards, cabbage, wild radish (*Raphanus raphanistrum*); worm-seed mustard (*Erysimum cheiranthoides*), ball mustard (*Neslia paniculata*), apetalous pennycress (*Lepidium apetalum*), pennycress (*Thlaspi arvense*), false flax (*Camelina sativa*), wild peppergrass (*Lepidium virginicum*), and shepherd's purse (*Capsella bursa-pastoris*). The seeds of one pot of each were thoroughly wet with a spore-suspension of *Phoma lingam* made from pure culture. Each of the pots contained approximately 100 seeds, except in the case of the wild radish where only 22 seeds were available and they were equally divided between the inoculated and control pots. The first infection appeared on the eighth day after planting. Ninety per cent of the collards and 75 per cent of the cabbages were diseased and three wild radish seedlings showed cotyledon infection. Five days later collards and cabbages were all dead and 9 of the wild radish seedlings were infected in cotyledons or stems. In the latter case the hypocotyl showed a discolored streak extending from the soil level almost

TABLE 3

Seed inoculation

PLANT USED	HOW INOCULATED	CONDITION ON 12TH DAY		CONDITION ON 17TH DAY		PER CENT INFECTION
		Inoculated plants	Control plants	Inoculated plants	Control plants	
Rutabaga	Seeds wet with spore suspension	Dwarfed and dark colored. Several shriveled at base	Twice the size of inoculated	1 alive but infected. Pycnidia	Good condition	100
Cauliflower	Seeds wet with spore suspension	Dwarfed, dark color. Stems shriveled with purplish color	Twice the size of inoculated	Abundant pycnidia on prostrate hypocotyls	3 damped off. Rest in good condition	100
Kohlrabi	Seeds wet with spore suspension	Dwarfed, dark color. Many shriveled at base	Twice the size of inoculated	All dead. Pycnidia on cotyledons and hypocotyls.	Good condition	100
Kale	Seeds wet with spore suspension	Dwarfed, dark color. Nearly all shriveled at base	Twice the size of inoculated	All dead. Pycnidia at base of hypocotyls	Good condition	100
Rape	Seeds wet with spore suspension	Dwarfed, very dark color. Several withered at base	Twice the size of inoculated	All dead but one. Pycnidia near base of hypocotyls	Good condition	98
Turnip	Seeds wet with spore suspension	Slightly smaller. All but 4 shriveled at base	Larger than control. Good condition	2 shriveled at base. Others dead	Good condition	100

Chinese mustard	Seeds wet with spore suspension	Few dwarfed. Others apparently normal	All vigorous	Dark spots on cotyledons with pycnidia. Several shriveled and dark streaks at base of hypocotyls	Good condition	50
Black mustard	Seeds wet with spore suspension	Several dwarfed. Some shriveled at base	Larger than inoculated	50 per cent shriveled or with cotyledons distorted. Some others discolored near base. Pycnidia on cotyledons and prostrate stem	Good condition	65
Charlock	Seeds wet with spore suspension	Several shriveled at base and cotyledons withered		Nearly all shriveled at base or with cotyledonous infection. Pycnidia on cotyledons. Prostrate hypocotyls		85
Radish	Seeds wet with spore suspension	Dwarfed. Many cotyledons show infection	Larger than inoculated. Some cotyledons slightly distorted	All shriveled at base. Pycnidia on cotyledons and prostrate hypocotyls	Vigorous	100
Garden cress	Seeds wet with spore suspension	1 slightly withered at base. 1 discolored streak at base	None	More than half shriveled at base. Pycnidia at base of hypocotyls		64
Peppergrass	Seeds wet with spore suspension	No seedlings	None	Few seedlings. No infection		

TABLE 3—*continued*

PLANT USED	HOW INOCULATED	CONDITION ON 12TH DAY		CONDITION ON 17TH DAY		PER CENT INFECTION
		Inoculated plants	Control plants	Inoculated plants	Control plants	
Stock	Seeds wet with spore suspension	Fungus growth on some cotyledons	None	50 per cent shriveled at base. Pycnidia on cotyledons and hypocotyls		50
Sweet alyssum	Seeds wet with spore suspension	All apparently healthy	None	Several infected. Pycnidia on cotyledons of 1 plant		10
Tumble mustard	Seeds wet with spore suspension	Several dwarfed. Some shriveled at base. Pycnidia on prostrate hypocotyls	None	Nearly all shriveled at base. Pycnidia		95
Hedge mustard	Seeds wet with spore suspension	Apparently healthy	None	Several shriveled and broken over. Pycnidia on prostrate hypocotyls		20
Shepherd's purse	Seeds wet with spore suspension	Seedlings just appearing above surface of soil	None	All healthy		

to the first leaves, and the base was beginning to shrivel. The lesions on the cotyledons had developed pycnidia. One worm-seed mustard plant had withered down and pycnidia were found on the hypocotyl near the cotyledons. None of the other species showed infection at this time or later. The controls remained healthy in all cases.

In May, 1914, the crucifers which had been tested under greenhouse conditions for susceptibility to *Phoma lingam* were planted in an infected plot in the pathological garden. To insure complete soil infection, small bits of diseased host tissue were generously distributed in the rows at the time of planting the seeds. Just as the tender seedlings were well out of the ground, a terrific hail-storm passed over the plot, nearly or quite destroying several of the species and damaging the others to such an extent that it seemed as if the experiment would fail. However, a sufficient number of plants survived to yield some positive, although very incomplete, results by May 27 when the last observation was made. The stocks, false flax, tumble mustard, wild peppergrass, cauliflower, and Brussels sprouts were all killed by the storm. Of the following, three to twelve plants were left in each case none showing infection: garden cress, worm-seed mustard, pennycress, ball mustard, hedge mustard, black mustard, new monstrous mustard, scarlet turnip radish, and white Strasburg radish. Charlock and Chinese mustard showed about 1 per cent infection on the remaining plants, and of fifteen white mustard plants, three were infected. Kohlrabi, collards, kale, rape, mammoth rock-red cabbage, red Dutch cabbage, and All Seasons cabbage showed abundant infection; turnip and rutabaga showed slight infection, and shepherd's purse, with a good stand, no infection.

From the preceding experiments, it is evident that *Phoma lingam* has a very wide range of hosts in the mustard family. Of the eleven genera tested, four, Capsella, Neslia, Thlaspi, and Camelina, were found to be resistant to the fungus, while the others, Brassica, Raphanus, Sisymbrium, Lepidium, Matthiola, Lobularia, and Erysimum, varied, in the order named, from almost complete susceptibility to nearly perfect resistance. All of the species of Brassica were readily attacked by the fungus in the seed inoculation experiments; in the field, even under very unfavorable conditions, all but one, *Br. nigra*, showed some infection. The two species of Raphanus proved very susceptible in the greenhouse, but showed no infection in the garden. The two species of Sisymbrium were also readily infected in the greenhouse tests, but, unfortunately, no field observations could be made. One of the three species of Lepidium proved susceptible in the greenhouse but no infection had occurred in the field when the last observation was made. The one species of Matthiola tested showed

relatively high susceptibility in the seed inoculations, but no data could be collected on the field experiment. Lobularia and Erysimum were only slightly susceptible in the greenhouse tests.

EXPERIMENTS AND OBSERVATIONS ON MOTHER SEED-PLANT INFECTIONS

The statement, that a certain lot of poor seed has been the means of introducing a particular disease into a new section of the country, or on to a newly cultivated portion of a farm, is frequently met with among the growers of various vegetable crops. This is especially true among cabbage growers in sections of Wisconsin, the black-leg being one of the diseases said to be thus introduced. Believing this statement to be true, at least as far as it relates to *Phoma lingam*, the writer carried out a series of experiments to test its validity.

During the last week of April, 1913, a number of mother seed-plants which had been grown at Racine on soil free from Phoma and which, so far as could be determined, were free from disease of any kind, were set out in the pathological garden. Inoculation experiments were begun on these plants as soon as the blossoms began to open. These were continued at irregular intervals through the season, until the seed-pods were fully formed and the seeds fairly well developed. Inoculations were made by spraying the plant thoroughly with an aqueous spore suspension made from pure culture of *Ph. lingam*, and then keeping it in a moist chamber, arranged to allow free access of air, for twenty-four to forty-eight hours. The moist chamber was secured in two ways. (1) A large candy tub, provided with numerous auger holes through the bottom, was inverted over the plant, or (2) an oblong wooden box, provided with air holes at the sides and with both ends open, was slipped down over the plant, and then capped with a large tubulated bell-jar. This was protected from direct sunlight, to prevent excessive rise of temperature within the chamber, by a screen of heavy burlap.

The first inoculation was made just as the lower outer blossoms of the raceme were beginning to open. The first distinct evidences of Phoma action were observed during the third week after inoculation, although many flowers had withered, and fallen prior to that time. The inoculated plant developed more slowly than did the untreated ones, and seemed much less vigorous. Part of this effect was probably due to the confinement in the damp chamber, but it soon became evident that fungus action was largely responsible for the trouble. Discolored areas appeared on the foliage leaves at the base of the flower axis and, gradually enlarging, resolved themselves into typical lesions with purplish well-defined borders and lighter colored slightly sunken interiors, which soon became dotted

with subepidermal pycnidia. Flower clusters at the tips of several young branches had shriveled up and the tender discolored flower parts showed abundant development of pycnidia just beneath the epidermis. The fungus passed gradually downward from these through the pedicels into the peduncles and, in many instances, into still larger flower stalks, shriveling and killing the tissues as it progressed. The advancing ends of such lesions were sharply marked by purplish borders, while the tissues back of these were lighter in color and contained numerous pycnidia. As a result of this progressive development, the discolored stump of a lateral branch was often observed still attached to the main axis at the middle of a lesion which was evidently widening on all sides, especially in the direction of the long axis of the stem. Lesions occurred very frequently upon the smooth, primary and secondary axes of the inflorescence where they were unusually conspicuous (fig. 4), their dark purple borders being in marked contrast with the light green color of the healthy stems. These lesions spread mainly in the direction of the long axis, but often one would widen sufficiently to nearly or quite encircle the stem. By the middle of the season, the central flower axis of the plant was thus cut off near the base before many of its seeds had matured.

FIG. 4. PHOMA LESION DOTTED WITH YOUNG PYCNIDIA ON THE FLOWER STALK OF CABBAGE SEED PLANT. NATURAL INFECTION

One week after the first experiment was started, two other plants were inoculated. The petals of many of the outer flowers had already fallen off and the pods were fairly well developed. A considerable number of young blossoms darkened and dropped off within a week after the moist chambers were removed; but, as in the previous experiment, unmistakable

evidence of infection was not observed until about two weeks later. The early infection was as already described for the first experiment.

The first infection observed on the young siliques occurred at the distal end, the organism apparently having entered the stigma or style, since these were the first parts to show discoloration and develop pycnidia. From such an invasion, in some cases the spread of the lesion was about equal on all sides of the pod, causing a shriveling of the entire distal end, a purplish more or less irregular band lying between the healthy and diseased areas. In other cases, the development was more rapid in a narrow strip of tissue along the suture, resulting in an elongated discolored streak. The shrinking of the tissues frequently caused a separation and twisting of the valves, which showed abundant pycnidia along their ruptured edges.

FIG. 5. PORTIONS OF CABBAGE SEED PODS SHOWING PHOMA LESIONS WITH PYCNIDIA NATURAL INFECTION

Infections also occurred at different points along the sutures and always gave rise to a long narrow lesion following the line of dehiscence. The middle region of such an area was generally of a lighter color than the margins and dotted with pycnidia.

Lesions also occurred at various places on the valves. They began as brownish or purplish spots which gradually enlarged, becoming light colored and sunken in the central areas which later produced numerous pycnidia. The brownish to purplish borders of these lesions were less clearly defined and less conspicuous than were those of the seed-stalks, the dark color shading more gradually into the lighter central region on one side and into the green of the healthy tissues on the other. They varied in shape from nearly circular to elongate oval or oblong in the direction of the long axis of the pod (fig. 5).

A few pods were found on which sub-epidermal pycnidia had developed in considerable numbers without the formation of distinct lesions. In surface view, these areas differed from the normal tissues by being just a shade lighter in color and in having the epidermis slightly more wrinkled and uneven.

Some cases were observed where the fungus had reached the silique by passing outward through the pedicel. Such infection usually resulted in early withering and death of the pod by cutting off its food supply.

The next inoculation was made when the earliest seed-pods were nearly or quite mature, but still green. The plant had grown so large that it was rather difficult to provide it with a suitable moist chamber, but the box and bell-jar were used as already described. Careful observation failed to discover any infection on this plant at any time.

Natural infection of mother seed plants

In November, 1913, two mother plants were taken from the storage cellar and transplanted into a ground plot in the greenhouse. Both

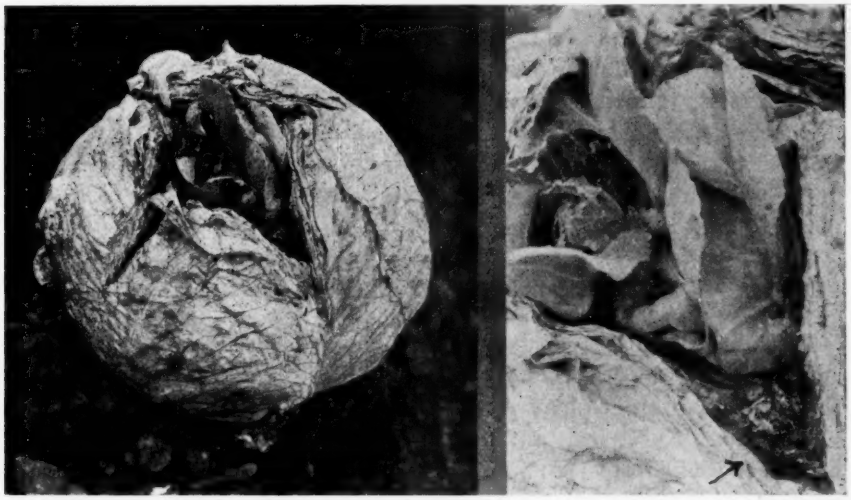

FIG. 6. At left, mother-seed plant with young flower stalks just bursting through top leaves. At right, central part of same on larger scale to show Phoma pycnidia developing at the angle indicated at the arrow.

plants showed Phoma infection on the stem below the head. One of them had been so badly diseased during the growing season, that it had developed a head not more than five inches in diameter. This plant died within two weeks. The other one had produced a comparatively good head and the

only infection observed was one small lesion (about 1 cm. diameter) on the stem near the base of the head. By the middle of December, this plant had put forth a healthy looking central seed-stalk two or three inches long. Three days later, on December 18, the old tissues, at the angles of the slits caused by the pushing out of the central bud, were abundantly dotted with mature Phoma pycnidia which discharged myriads of spores whenever sufficient water was applied to dampen them (fig. 6). These were so located that many of the young developing parts necessarily came in direct contact with them, and more remote parts of the flower axis could hardly escape infection in the early stages of growth by the splashing of the spore laden water out to them.

The fungus had apparently passed from the point of original infection in the stem, up through the older leaves of the head to the cut surface of the leaves where it began to fruit immediately. This assumption harmonizes with the facts observed in storage, namely, that the fungus does pass upward through the central axis and outward through the leaf petioles, producing abundant pycnidia on reaching the surface.

This is undoubtedly a very important method of mother seed plant inoculation and probably is the chief means by which the fungus gains access to the flowering parts. If, as Ritzema Bos suggests, the fungus mycelium may remain in the stem in a semidormant condition through a considerable portion of the growing season and then resume development in storage, it is conceivable that infected plants might be held over winter and be transferred to the seed plots the following spring, where the disease would develop abundantly. This seems to be what happened in the case of the mother plants which were observed at Onalaska.

Observations on mother seed plants at Onalaska

In the fall of 1912, R. B. Wilcox selected a number of well-headed cabbage plants from a Phoma infected field at Onalaska. It was hoped that these plants, or at least some of them, since they showed no infection, would prove to be resistant to the disease. They were stored by the Onalaska Pickle and Canning Company and replanted during the first week of May, 1913.

June 20, when the writer visited the plot, 60 per cent of the plants were either dead or broken over at the surface of the soil or a few inches above it. Death had probably resulted from a combination of causes. Many of the plants showed distinct Phoma action at the base of the stem and on the roots, and lesions, with pycnidia, occurred on the young leaves and shoots. The living plants were all infected with Phoma, some of them very badly. Typical lesions were abundant on leaves and flower-

stalks. On the latter, they were especially conspicuous, the very dark purple borders being in marked contrast with the white interior of the lesion on one side and the light green of the healthy stem on the other (fig. 4). They were elongated in the direction of the long axis of the stalk and some of them had widened sufficiently to almost encircle it. Others on smaller lateral branches had apparently eaten through the tissues of the stem so that it had broken off, leaving only an infected stump attached to the larger axis. The withering and death of the young foliage leaves, due to infection, was especially conspicuous. A number of infected pods were also found. In some cases the fungus had apparently entered through the flower and passed on to the end of the pod which was shriveled and bearing pycnidia. Other pod infections had evidently started at some point on the suture along which the fungus developed in both directions, producing a long narrow lesion. In some of these the shrinking of the diseased tissues had caused the valves to separate through the lesion, and pycnidia had developed on their edges. The few lesions found on the surface of valves were like those on the seed-stalks, except perhaps that the purple border was slightly more diffuse. Infected flowers were also found whose parts were shriveled, discolored, and producing pycnidia.

This field was visited again six weeks later, but unfortunately some ill-advised farm hand had been over the ground with a mower and cut heads from all of the seed-plants so that further observations were impossible.

Isolation of Phoma lingam from naturally infected seed

On the second of August, 1913, a green, almost mature, silique on one of the artificially infected plants was observed to contain a Phoma lesion near the middle of one valve directly over a seed. The lesion was small, slightly depressed, and lighter colored in the interior, but had not yet developed pycnidia. This pod was carefully removed, immersed in bichloride of mercury solution, 1: 1000, for five minutes, and rinsed in sterile water. With flamed forceps one valve was carefully turned back, beginning at the pedicel end; and as each seed was exposed it was removed with sterile forceps and placed on a culture plate of potato hard agar. Three seeds were thus plated on separate plates, the third being taken from directly beneath the lesion. In external appearance, so far as could be observed under the conditions, this seed did not differ from the other two. The area of the valve just over it, however, was darker in color than the surrounding tissues, but could not be observed to be ruptured in any way. Three days later hyphae could be seen pushing out from the seed taken from beneath the lesion. On the fourth day, transfers were

made to agar slants, and by the seventh day, pycnidia had developed on the plate and in the tubes. On the twelfth day, the control seeds were still sterile.

Several infected pods containing mature seeds were selected early in August and stored until the last of November when the seeds were carefully removed from beneath the lesions of the valves and examined. All of them were perceptibly darker in color on the side toward the lesion, and several were considerably shrunken. Five of them showed tiny tufts of white mycelium projecting from the seed-coat, and the cotyledons of one

FIG. 7. PHOMA INFECTED CABBAGE SEEDS. × 4

of these were exposed through the seed coat and were greenish in color (fig. 7). December 12, 1913, two of these seeds were immersed in bichloride of mercury solution, 1: 1000—the first for three minutes and the second five minutes—washed in sterile water, and plated on potato agar. The first seed was somewhat darkened in color on one side and very slightly shrunken, but it did not show any marked variation in appearance from the normal; the other was considerably shriveled and showed tufts of hyphae at several places on the surface. On the third day, fresh mycelium could be seen projecting from both seed-coats; twelve days later, pycnidia developed. In each case the fungus was identified as Phoma by

inoculation of healthy cabbage plants. May 2, 1914, four other seeds were treated as above described, except that two of them were immersed in the bichloride solution for six minutes and the other two for ten minutes. Three of the seeds were badly shrunken and two of these showed white tufts of mycelium at various places on the seed coats. Fresh mycelial threads could be seen protruding in all directions from the coats of these seeds twelve hours after plating. Two of them yielded pure culture of *Ph. lingam*, the other had some bacterial contamination. The fourth seed appeared normal except for a slightly darker color of the seed coat on one side. It germinated on the third day after plating and at the same time mycelial threads could be seen projecting out from the seed coat,

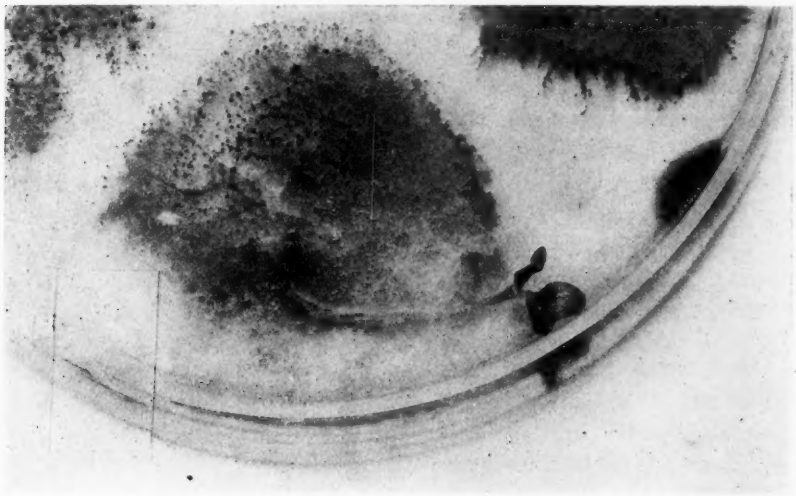

FIG. 8. CABBAGE SEEDLING ON AGAR CULTURE PLATE DEVELOPING PHOMA
Seed had been immersed for ten minutes in 1:1000 solution of mercuric chlorid.

which was lying loose from the seedling. This also yielded a pure culture of *Ph. lingam*, which attacked the young seedling, and by May 25, pycnidia had developed on roots and hypocotyl (fig. 8).

Diseased plants from naturally infected seed

Early in August, seeds from the artificially inoculated seed plants were thrashed out by rubbing the infected pods and stalks between the hands, and the whole mass stored in a glass jar. November 25, they were separated from the chaff and planted in a ground plot in the greenhouse, which, so far as was known, had never had any opportunity to become

infested with Phoma, and which, as a further precaution, had been thoroughly steam-sterilized two days before. Phoma infection was observed fifteen days after planting and by the end of the third week, thirty-two badly infected seedlings, with well developed pycnidia had been removed from the plot. From this time on the disease became more and more apparent but no further counts were made of the infected plants.

FIG. 9. CABBAGE SEEDLING SHOWING PHOMA-INFECTED COTYLEDONS
Grown from naturally infected seed

Infection apparently began either at the base of the hypocotyl or at some point on the cotyledons and in a few instances at both of these places. When the entrance was at the base, shriveling of the hypocotyl also began at that point and progressed upward, pycnidia developing always in the region longest infected. From cotyledon infection (fig. 9) the progress of the fungus was in the reverse direction as shown by the shriveling of tissues and development of pycnidia. Some cases were observed in which

the infection appeared to come directly from the old seed coat which had been carried up on the cotyledon and often remained attached to its apex until it was badly shriveled and producing fruit bodies. It seems quite probable that in such cases the fungus may have developed first saprophytically on the seed coat and from this substratum passed on to the host. This phenomenon has frequently been observed in various seed inoculation experiments.

A probable means of secondary infection was also observed in this plot. The young diseased seedlings were frequently found to have become attached by the cotyledons to the leaf or stem of a healthy plant nearby. Where the sick seedling adhered to the stem, infection was likely to result only at the point of contact between the diseased and healthy tissues; but when it became attached to the young leaf it was carried upward by the growth of the normal plant and not only infected the leaf to which it adhered, but became a source of spore distribution to a wider circle of plants because of its exposed and elevated position. Recently developed pycnidia, such as occur on the cotyledons and hypocotyls of young seedlings, discharge their spores immediately upon the application of moisture so that the condition favorable for spore dissemination by spattering of water drops also provides numerous spores.

SEED BED AND FIELD EXPERIMENTS

During the spring and summer of 1913, a series of experiments was carried out to determine (1) whether *Phoma lingam* lives in the soil from one year to the next, (2) how it is carried over, (3) whether the disease may be carried on the seedlings from an infected seed-bed to the field, and (4) whether healthy seedlings may become infected when transplanted into diseased soil.

On April 21, flats of soil which had produced diseased plants several months before and which had been free from living plants during the winter, were sown with cabbage, cauliflower, kohlrabi, and Brussels sprouts. One flat of clean soil planted with cabbage served as a check. They were transferred from greenhouse to garden on the ninth day and many of the tender seedlings were killed by frost, and all were more or less stunted. The check plants were larger and more vigorous than those in infected soil. Phoma lesions appeared on stems and leaves in all infected flats within three weeks. No record was made of the percentage of infection.

On May 16, soil, with the diseased cabbage stumps it contained, was collected from a Phoma-infested field at Onalaska and shipped to Madison. June 1, four plots were prepared by digging away the surface soil to a depth

of two or three inches and filling the space with the sick Onalaska soil from which the stumps had been removed.

These were immediately planted with cabbage seed which had been treated for fifteen minutes with a 1:200 solution of 40 per cent formaldehyde and left in the direct sunlight in the greenhouse to dry. The treatment was apparently too drastic since none of the seeds germinated, so the plots were resown fifteen days later. A fifth plot was prepared by cutting the diseased stumps from the Onalaska soil into bits and mixing them with clean soil. During the first month, no diseased plants were observed in the soil from which the stumps had been removed, while that to which the stumps had been added gave 100 per cent infection. Later, however, infection appeared at various places in the four plots, but did not become general. Much of this may have come from the fifth plot since the beds were so close together that the outer leaves of many of the plants were in contact. This experiment indicated that the greater part of the infectious material was removed from the soil with the stumps which were taken out only two weeks before planting, and it unquestionably shows that *Ph. lingam* does live over in the diseased host tissues from one season to the next.

Late in April, a seed bed was started on a plot in which a number of plants had been killed by black-leg the previous season. The disease began to appear in the bed three weeks after planting but did not become general until the seventh week. During the last week in June, 105 plants were selected from this bed and transplanted to a field, which, so far as could be ascertained, had never grown cabbage. A week later, 81 other plants were also transplanted to this same field. No infection was detected on any of the transplanted seedlings, but no precautions were taken to prevent contact with diseased plants or distribution of spores by handling.

Lesions began to appear on the plants in the field a few weeks after transplanting, both leaves and stems being attacked. On August 8, when final observations were made on the field, it was found that of the 186 plants set out, 128 were either dead or so badly diseased that they had failed to produce any kind of head. Many of them had been attacked at or below the surface of the soil so that nothing remained of the root system but a blackened stump. A few had put out new roots above the lesion and were making a very feeble growth. Others were girdled at the surface of the soil and had broken over. A few which had been girdled were still standing and the root system had apparently escaped infection.

Several rows of seedlings transplanted from a clean seed-bed to this same field served as controls. Phoma was not observed, except in two cases where a slight leaf infection—clearly secondary—had occurred in the row adjoining that of the diseased plants.

The above results clearly show that the black-leg organism is readily transferred with the plants from the infected seed-bed to the field, and that control of the disease is impossible unless the seedlings are grown on clean soil.

During the first week of July, 25 seedlings from a clean seed-bed were transplanted to a Phoma-infected plot in the pathological garden. The weather was so hot and dry that the plants were unusually slow in adjusting themselves to the new conditions, and this period of weakness seemed to offer a very favorable opportunity for the attack of the fungus. Two weeks after transplanting, all of the plants showed infection on leaves and stems, and by the end of the fifth week, all of them had been killed. Seedlings left in the seed-bed, as well as a few hundred which were transplanted to clean soil, all of which remained healthy, served as checks, proving that infection took place after transplanting.

DEVELOPMENT OF THE DISEASE IN STORAGE

Under the head of Krebsstrünke, Ritzema Bos (14) describes a phase of the black-leg disease which is met with only in storage. At harvest time, plants which show no external evidence of infection are often found to contain small dark spots in the vascular ring which harbor the mycelium of the fungus. During late winter, when store-rooms become warm and moist, these spots enlarge irregularly, sometimes being confined to the stalk, but more frequently extending to the leaves, usually attacking the outer ones first. From the axil of the leaf, the discoloration spreads to the veins which frequently become so blackened as to suggest the presence of *Ps. campestris*. The leaves are progressively cut from the stem, the outer ones first and are often covered with a flocculent mycelium producing abundant pycnidia.

NATURAL INFECTION

The storage phase of the disease is of minor importance in Wisconsin, hence the opportunity to study it has been limited. However, some observations corroborating those of Ritzema Bos have been made. In the autumn of 1913, 24 mature cabbage plants were taken from a Phoma-infected field and put into the root cellar of the pathologium. All of them showed slight Phoma infection, either on the stem below the head or on the leaves. The cellar was damp and poorly ventilated and the temperature soon rose sufficiently to provide admirable conditions for the development of fungi. After a few weeks, it was found that the lower outer leaves of many of these plants had fallen away from the stem, which,

in some cases, was also considerably shrunken, especially in the region of the old Phoma lesions. The cutting off of the leaves from the central axis gradually progressed upward toward the central bud, and by the

FIG. 10. PHOMA INFECTION IN THE SPRING ON STORED MOTHER SEED HEAD
Note that the leaves show killed areas and premature loosening from the stem.

middle of January, a more or less funnel-shaped opening had thus formed around the stem and extended almost to the middle of the head (fig. 10). Reddish-brown pycnidia developed abundantly on all cut surfaces and

beneath the epidermis of the outer leaves, and a dense whitish to grayish layer of mycelium was frequently observed covering the moister surfaces. In most cases, it was clearly evident that the old stem lesion was the point of initial infection; in others where no stem lesions were observed, it is probable that the fungus was present in the tissues, and although not sufficiently active to produce a lesion on the developing host, had been able to vigorously attack the dormant tissues when brought into more favorable conditions. The mycelium was abundant in the pith of the stem, where it was both inter- and intra-cellular. The walls of the invaded cells were darkened and the protoplast nearly or quite destroyed. From this central axis the fungus passed outward through the petioles, in the region of the vascular bundles, into the leaf tissues where it not only followed the veins, but spread also to other parts of the leaf. The invaded tissues became so dried and shrunken that, when compressed between the hands, they seemed to be of the consistency of loose, soft paper. In a few cases, a soft rot developed in connection with the disease, but this was probably due to saprophytic organisms, since they were always present. Pycnidial development was observed to take place always on exposed surfaces and in connection with the more nearly exhausted tissues.

ARTIFICIAL INOCULATIONS

November 28, four healthy cabbage heads were inoculated with pure culture of Phoma, three by cutting the central axis at the base of the head and inserting a portion of the fungus and the other by turning back the edges of the leaves and putting the culture between them. Infection resulted in all cases, but the development was much slower than with the naturally infected plants, no observable change having taken place until nearly three months later, when the mycelium had spread to some of the outer leaves and development of pycnidia had begun. One head was cut open and it was found that the fungus had passed up through the pith for a distance of about 2 inches and the cell walls were much darkened. Portions of tissue at the advancing margin of the fungus were removed aseptically and plated on potato agar. From these, pure cultures of the fungus were secured proving it to be advancing independently of any other organism.

About the middle of March, a helper about the greenhouse inadvertently cut away the diseased tissues from all but one of the inoculated heads, thus destroying further observations. In this head, the fungus had become so widely distributed that it continued to develop and by the second week in April showed a condition like that of the naturally infected plants. The leaves had separated from the central axis and pycnidia were abundant on nearly all exposed surfaces.

EXPERIMENTS WITH CONTROL MEASURES

Various methods, such as disinfection of seed (8, 6), spraying of seed beds with fungicides (9, 8), sterilizing of seed bed with heat (9, 6), sanitation and crop rotation, have been suggested for the prevention of the black-leg disease of cabbage, yet very little experimental data has been collected which bears directly on this important problem. The writer has carried out some experiments along lines which have been suggested by previous workers, and by his own studies.

Seed treatment

Although cabbage seed treatments have been recommended by different writers (8, 6), they appear not to be based upon extensive experimental data, and there is no unanimity of opinion as to just which treatment to apply. Two purposes of the present studies have been to determine: (1) the most suitable fungicide, and the most satisfactory method of application from the standpoint of seed germination, and (2) whether such treatment is sufficiently drastic to insure elimination of Phoma as it occurs in connection with the seeds.

A series of preliminary treatments was carried out, using the following fungicides in different strengths of solution as indicated: 40 per cent formaldehyde in four dilutions by volume—1: 100, 1: 200, 1: 250, and 1: 300; bichloride of mercury in two strengths of solution—1 gram to 500 cc. of water and 1 gram to 1000 cc. of water; and potassium permanganate in two strengths of solution—15 grams to 1000 cc. of water and 20 grams to 1000 cc. of water. Germination was tested by placing the seeds in moist chambers. Different periods of exposure were tried for the different fungicides ranging from five to thirty-five minutes in the stronger and from fifteen to forty-five minutes in the weaker solutions. Unfortunately, the seeds used in these tests were so low in vitality, and the moist chamber method of germination was so unsatisfactory, that the results obtained were not considered worthy of record. They did indicate, however, that the best results were likely to come from the use of 40 per cent formaldehyde at the 1: 100 and 1: 200 dilutions, or from the bichloride of mercury in the 1: 500 and 1: 1000 strengths; also that the period of treatment need not be less than fifteen minutes for either of the solutions.

In all later trials, formaldehyde and bichloride of mercury were the only fungicides used, and in the dilutions above indicated. The seeds were separated into equal lots and each lot was tied in a little square of muslin for convenience in handling, and then immersed in the fungicide. In most cases, two lots of seed were treated for the same length of time,

after which one was washed and the other allowed to dry without washing; but this procedure was varied in some cases by treating three lots of seed, washing one, and allowing the other two to dry without washing—one slowly, the seeds being left in the muslin bag in a heap, and the other quickly by spreading the seeds out; and occasionally like lots of seed were treated for dissimilar periods in order to emphasize the different effects of the fungicides upon them. Germination tests were made in steam sterilized sand or soil.

Tables 4 to 10 show the results of these tests.

TABLE 4

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION		
					Washed	Unwashed	Untreated
40 per cent formaldehyde..	1:100	5	2	100	79*	71*	95
	1:100	10	2	100	79*	60*	
	1:100	15	2	100	83	70	
	1:100	20	2	100	68*	68*	

* A damping off fungus interferred with germination.

TABLE 5

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION		
					Washed	Unwashed	Control
40 per cent formaldehyde...	1:200	15	2	100	75*	73*	94
	1:200	20	2	100	90	87	
	1:200	25	2	100	90	89	

* Damping off fungus interfered with germination.

TABLE 6

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION	
					Washed	Control
40 per cent formaldehyde....	1:100	15	1	300	85	95
	1:100	25	1	300	88	

TABLE 7

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION			
					Washed	Unwashed		Control
						Dried quick	Dried slow	
40 per cent formaldehyde..	1: 200	25	3	200	93	83	86	95
	1: 200	35	3	200	89	78	86	95

TABLE 8

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION		
					Washed	Unwashed	Control
40 per cent formaldehyde..	1: 200	25	1	300	88		95
	1: 200	45	1	300		80	

TABLE 9

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION			
					Washed	Unwashed		Control
						Dried quick	Dried slow	
Mercuric chlorid ..	1: 1000	25	3	200	89	89	87	95
	1: 1000	35	3	200	89	84	82	

TABLE 10

FUNGICIDE	STRENGTH OF SOLUTION	NUMBER OF MINUTES EXPOSED	NUMBER OF LOTS TREATED	NUMBER OF SEEDS PER LOT	PERCENTAGE OF GERMINATION		
					Washed	Unwashed	Control
Mercuric chlorid....	1: 1000	10	2	100	90	80	95
	1: 1000	15	2	100	95	89	95
	1: 1000	20	2	100	94	91	95
	1: 1000	25	2	100	88	89	95
	1: 500	15	2	100	86	96	95
	1: 500	20	2	100	88	85	95
	1: 500	35	2	300	92	80	95

Comparisons of results as given in the preceding tables, 4 to 8 inclusive, indicate (1) that formaldehyde in the proportions of 1 cc. of 40 per cent formaldehyde to either 100 or 200 cc. of water does not materially reduce the germination of cabbage seeds when they are immersed in it for periods of from fifteen to twenty-five minutes, and even a little longer in the case

of the weaker solution, (2) that the germination is higher after the weaker solution, (3) that seeds washed after treatment give a higher percentage of germination than do unwashed ones, and (4) that the rate of drying does not materially affect the amount of germination. The tables, however, do not bring out some of the most important facts in connection with germination. It was observed that although the final percentage of germination, as indicated by the tables, did not differ materially, for the different strengths of solution and different periods of treatment, the rate of germination was decidedly interfered with. The longer periods of treatment, especially with the stronger solution, caused retarded and uneven germination and in the more extreme cases, the seedlings showed considerable distortion. This phenomenon was especially marked in the case where seeds were treated for forty-five minutes in the 1:200 solution and planted without washing. Retarded and uneven germination and less vigorous seedlings frequently resulted in cases where longer exposures to the fungicide were not followed by a thorough washing of the seeds.

The data collected point to the conclusion that cabbage seeds may safely be immersed in a 1:200 solution of 40 per cent formaldehyde for a period of twenty to twenty-five minutes without any material reduction in germination or loss of vitality provided they are washed immediately after removal from the fungicide.

From tables 9 and 10, it appears that the percentage of germination in cabbage seeds is not materially reduced by treatments of twenty to thirty-five minutes with either of the solutions of bichloride of mercury used, and that the washing after treatment is less important than in cases of formaldehyde treatment. However, the unwashed seeds from the longer exposure did germinate unevenly, and the very young seedlings appeared slightly less vigorous than when washing followed the treatment with the fungicide.

It appears, therefore, from the above results, that from the standpoint of seed-germination after treatment, the two fungicides used are equally satisfactory as seed disinfectants. However, from the point of view of practicability and ease of application, the formaldehyde treatment is more desirable for the following reasons: (1) the formaldehyde solution may be made up with any ordinary tap or well water, while the bichloride of mercury must have water practically free from organic or inorganic matter to prevent its precipitating out in some insoluble compound, (2) the formaldehyde solution is more easily prepared by the farmer in the quantities needed than is the bichloride of mercury solution, and (3) there is much less possibility of injury to the manipulator with the formaldehyde than with the bichloride of mercury.

Not having a sufficient number of Phoma-infected seeds to justify direct tests of the value of the fungicides, an attempt was made to determine this matter indirectly. Two methods were employed: (1) pycnidia still imbedded in the soft tissues of the host were immersed in the fungicide solution and then washed in sterile water and the spores placed on slides in a moist chamber to germinate, and (2) the spores were treated directly by fixing them to a slide with a smear of egg albumen and then covering them with the fungicide for the desired period, after which they were washed in water and put into the moist chamber to germinate. In all cases, germination was determined by microscopic examination.

Numerous trials demonstrated that the shortest period of exposure of pycnidia to a 1:200 solution of 40 per cent formaldehyde that was sufficient to kill all of the contained spores was twenty-one minutes. With the 1:1000 bichloride of mercury solution, slight germination was secured after twenty-one minutes; however, a sufficient number of trials was not made to accurately determine this point.

With naked spores, it was found that direct exposure to either of the fungicides for two to three minutes was sufficient to kill them.

These tests indicate that a twenty-one minute exposure of the seeds to the formaldehyde solution (1:200) would be sufficient to insure sterilization of all pycnidia and spores which might be adhering to their surfaces. This period of treatment comes well within that found in the previous experiment to be most satisfactory from the point of view of vitality and germination of the seeds, and appears to offer all that could be desired for surface disinfection. However, it does not take into account the fungus mycelium which, in many cases, has penetrated the seed coat and even entered the cotyledons. In such cases much more drastic measures will probably have to be resorted to in order to insure complete sterilization.

This assumption is borne out by a single test made with three infected seeds. They were immersed in a 1:200 solution of 40 per cent formaldehyde for twenty-one minutes and after washing in sterile water were transferred to a plate of potato agar. The second day after plating, hyphae could be seen projecting from the seed-coat of one seed and on the following day mycelial threads were developing from each of the others. All of them yielded pure cultures of *Ph. lingam*.

Control by Bordeaux mixture

To test out Bordeaux mixture as a means of Phoma control in the seed-bed, according to the recommendations by Manns (8), a seed-bed was selected at Onalaska on a field so badly infected that the 1912 crop was an entire failure. On May 16, 1913, the plot was seeded with the following

named varieties of cabbage: Short Stem No. 4, Bismarck, Henderson's Succession, Volga, All Seasons, and Early Summer. Sixty square feet of the west end of the bed was immediately sprayed with Bordeaux mixture (4:4:50 formula) at the rate of one gallon to ten square feet. The west half of this area was given two other like treatments, one June 7 and the other June 21, just before transplanting. One portion of the bed had thus received three treatments with Bordeaux, another had received one treatment and a third was untreated.

By August 6, considerable infection had occurred in both the treated and untreated portions of the seed-bed, but was most abundant in the untreated areas, being 80 to 90 per cent. In the portion which had received one treatment, it was 40 to 50 per cent and where the three applications of Bordeaux had been made, it was only 25 per cent. The infection was mainly in the stem, the lesions usually beginning near the soil level.

From the above results, it appears that although Bordeaux mixture, as applied, does perceptibly reduce the percentage of infection in the seed-bed, it does not control the fungus under Wisconsin conditions and, therefore, cannot be recommended for adoption by the growers.

Removal of cabbage refuse from soil by screening

A previous experiment having indicated that *Phoma lingam* does not live over in the soil for any considerable period of time independent of the host tissues, it was decided to test the question experimentally. November 1, soil was taken from infected garden plots into the greenhouse and, after carefully picking out the stumps and larger portions of diseased cabbages, a part of it was passed through a fine-mesh screen. Then to further remove bits of diseased tissue, a part of this screened soil was passed through a screen of still finer mesh.

November 15, flats were prepared and planted with cabbage as follows: In flat 1, the soil had been twice screened, in flat 2, it had been once screened, in flat 3, it consisted of the coarser particles separated out by the first screening. Flat 4 contained soil from which the coarser parts of the diseased stumps had been picked out by hand; flat 5 was filled with soil containing diseased stumps just as it was taken from the garden. A damping-off fungus became so vigorous and destructive shortly after planting that it was necessary to re-seed the flats. The second planting was more successful than the first, but it was not entirely satisfactory, since the seedlings were again attacked by the damping-off organism, which killed many of them outright and so weakened many others that they never recovered anything like normal vigor. The light relations were also bad, so that the plants which escaped the damping-off fungus made only a very feeble growth. Four months after seeding, the plants were

removed from the flats and examined individually for Phoma infection. The results are shown in table 11.

TABLE 11

FLAT	NUMBER OF PLANTS	CONDITION OF SOIL	NUMBER OF PHOMA INFECTED PLANTS
1	100	Twice screened	None
2	100	Once screened	None
3	95	Consisted of screenings from flats 1 and 2	24
4	40	Coarser parts of diseased stumps removed	6
5	*20	With diseased stumps as it came from field	None

* All but two plants attacked by damping off organism. Two were badly clubbed with *Plasmodiophora brassicae*.

Three days after the above counts were made, the flats were again planted with disinfected cabbage seed. A damping-off fungus attacked the young seedlings as soon as they appeared, but with less vigor than in the previous experiment. A few weeks later when the greenhouse temperature began to rise perceptibly—due to the bright sunlight during the day—many of the plants were observed to be yellowing, and many of them soon dried and shriveled in an erect position. This condition obtained to some extent in all of the flats, but was especially noticeable in 4 and 5. Some of these infected plants were plated out and a Fusarium was isolated from them.

May 15, the plants were removed from the flats and examined for Phoma. Flats 1 and 2 contained twice as many plants as did either of the others. Many of them had the "Yellows" disease, but Phoma infection could not be detected. One Phoma-infected plant was found in flat 3 and none in either 4 or 5, where most of the plants had been killed by Fusarium.

Two flats of screened soil and two containing the diseased soil still mixed with infected stumps were left outside during the winter. March 25, they were taken into the greenhouse and planted with cabbage seed which had been disinfected as described above. Three weeks after planting, seedlings infected with Phoma were found in each of the flats of unscreened soil, but no evidence of the fungus could be discovered in either of the other flats.

The above experiments, although rather unsatisfactory because of complications introduced by the presence of other parasitic fungi, have, nevertheless, furnished much evidence that Phoma persists in the soil only in connection with the host tissues. No case of infection was found in either of the flats containing screened soil, but it was observed in all of the unscreened flats, except flat 5 of the first experiment, and flats 4 and 5 of the second experiment. The low percentage of Phoma infection in some of

the unscreened flats, and total failure of infection in others was probably due to the extreme virulence of the other parasitic fungi, which attacked the seedlings earlier and rendered them less desirable for Phoma.

Burial of infected stumps in clean soil

In October, 1913, four boxes, 5 to 8 feet long by 10 inches wide and having a depth of 6, 8, 10, and 12 inches, respectively, were set into the ground with their tops projecting just enough to prevent flooding by surface water and filled by putting into them first a layer of clean soil about 2 inches deep, then a layer of badly infected cabbage stumps and on top of these enough soil to round up well at the top. The stumps were thus buried in the different boxes to a depth of 4, 6, 8, and 10 inches, respectively. April 17, 1914, these boxes were removed to the greenhouse and planted with cabbage seeds which had been disinfected by immersing them for twenty-three minutes in a 1:200 solution of 40 per cent formaldehyde.

June 1, they were carefully examined and one diseased seedling was found in the box where the infected stumps were buried to a depth of 4 inches. In this case it is questionable whether the infection came from the buried stumps or not, since a diseased plant bearing pycnidia was let fall on the box at this point when the seedlings were about three weeks old. The infection also occurred at a point on the stem some distance above the surface of the soil where it could hardly have taken place had the fungus passed up from the soil.

Twenty-five seedlings were taken at random from each of these boxes and transplanted to Phoma-free soil in the field. None of these plants showed infection at any time during the season.

The boxes were kept in the greenhouse during the summer and the plants were carefully examined several times during September. No infection was discovered.

The above results indicate removal of diseased tissues and deep fall plowing as two important factors in the control of black-leg in fields already contaminated with Phoma.

SUMMARY

The black-leg disease of cabbage is widely distributed in the State of Wisconsin, the damage caused by it varying from the loss of a few heads to almost entire destruction of the crop.

Plants may become infected in all stages of growth, but are most susceptible during the seed-bed period, and the period of weakness immediately following transplanting. In the earliest stages, the diseased seedling is distinguishable from others by its smaller size and darker green color.

About the tenth day after planting, it shrivels at the base, has a dull grayish-white color and is sparsely dotted with minute subepidermal swellings. Occasionally a slight purplish discoloration may be noted just in advance of the shrunken area. The tissues collapse very rapidly at this stage, giving the impression of damping-off. Infection frequently begins in the cotyledons which shrivel somewhat and take on a dull dark green color.

The disease becomes most conspicuous in the seed-bed shortly before transplanting. Infected plants are lighter in color, less vigorous, and show whitish sunken areas on the stems near the surface of the soil. These areas, at first small, rapidly enlarge until they frequently encircle the stem and extend from leaves to roots. Pycnidia are seen as minute black dots scattered over the sunken area. The lateral roots are often missing and the softer tissues of the tap-root are destroyed. Lesions may occur on the leaves at this time, but are not common.

The disease appears in the field shortly after transplanting. Leaf infection is common, the lesions frequently becoming so numerous and extended as to interfere seriously with leaf functions. These are depressed, whitish areas dotted with dark-colored pycnidia and surrounded by more or less purplish borders. Stem infection may result in a much elongated lesion extending nearly or quite from roots to leaves and penetrating deep into the xylem tissues; or it may girdle the stem and allow the head to break off at the surface of the soil. Frequently nothing is left of the root system but a blackened stump. In other cases, secondary roots develop above the lesion and the plant makes a feeble growth throughout the season but seldom, if ever, produces a marketable head.

Purpling of the foliage leaves cannot be relied upon as a characteristic symptom of the disease in Wisconsin, since (a) it does not consistently follow the attack of the fungus, (b) it has been found to follow root-degeneration from excessive water, also decay due to the club root organism, and (c) it has been found to occur only on the leaf surfaces exposed to the direct rays of the sun.

Two types of pycnidia have been found developing on plants attacked by the black-leg disease of cabbage. Pycnidia of Type No. 1 develop subepidermally and on cut surfaces of the host either in field or storage; those of Type No. 2 develop on the exposed woody tissues in early spring.

In young infections, the mycelium follows the intercellular spaces and passes between adjacent cells. In later stages, it penetrates the cells in all directions, causing collapse and drying of the tissues.

The fungus causing the disease was first described by Tode (20) in 1791, as it occurred on dried stems of *Brassica oleracea rubra*, and given the name of *Sphaeria lingam*, which was recognized by Persoon (10) and Fries (5). In 1849, Desmaziere (4) transferred it to the genus Phoma making the name *Phoma lingam* (Tode) Desmaz.

Phoma siliquastrum Desmaz. (4), *Aposphaeria brassicae* Thüm. (18), *Phoma oleracea* Sacc. (17) and *Phoma brassicae* Thüm. (?) are names applied to this same fungus as it occurred under varying conditions, and there is considerable evidence for putting *Phoma napobrassicae* Rostr. in the same class.

The fungus occurring on *Melilotus alba* and designated as *Phoma oleracea* Sacc. (19) does not produce infection when inoculated into the cabbage and should be transferred to a different species of Phoma.

Phoma lingam (Tode) Desmaz. is a vigorous pathogene on the cabbage, infection resulting readily from (a) wounding, either before or after application of the spore suspension, (b) spraying, without wounding, (c) wetting of roots in spore-suspension at time of transplanting, (d) wetting of seeds with spore-suspension made either from pure culture or from old pycnidia on over-wintered stumps.

The incubation period of the fungus varies from seven to twenty-eight days; pycnidia developing usually in about two weeks. Artificial seed-inoculation does not reduce the percentage of germination, but it does produce very early infection of the seedlings which results in their slower rate of growth and smaller size.

Phoma lingam (Tode) Desmaz. has a much wider range of hosts in the Cruciferae than has hitherto been attributed to it, having been successfully inoculated into cabbage, cauliflower, Brussels sprouts, kohlrabi, kale, rape, collards, red Dutch cabbage, mammoth rock-red cabbage, rutabaga, turnip, white mustard, black mustard, Chinese mustard, charlock, cultivated radish, wild radish, tumble mustard, hedge mustard, stock, sweet alyssum, and worm-seed mustard.

Wild peppergrass, shepherd's purse, ball mustard, apetalous pennycress, pennycress, and false flax were found to be non-susceptible to the fungus under the conditions imposed.

Phoma lingam readily attacks all parts of the raceme of the mother seed-plants of cabbage when artificially inoculated by spraying with spore-suspension from pure culture.

Phoma lingam attacks mother seed-plants under normal field conditions infecting seed-stalks seed-pods, and seeds. Such an infection was observed at Onalaska, Wisconsin, in 1913.

Initial infection of the raceme of mother seed-plants takes place by the fungus passing from the point of last year's infection up through the head, and producing abundant pycnidia at the upper surface in the angles caused by the rupture of the old leaves by the pushing through of the central seed-stalks. The spores discharge readily whenever wet and get on to the young growing parts by direct contact, or by splashing of the water, or by insect transfer.

The fungus penetrates the seeds, causing them to shrivel and darken perceptibly. Frequently white tufts of mycelial threads are visible on the surface.

The fungus is carried over in the seeds, probably in the form of dormant mycelium and has been isolated from them after (a) removal from the pod under aseptic conditions, (b) exposures of 3, 5, 6, and 10 minutes, respectively, to a 1:1000 bichloride of mercury solution, and (c) immersion in a 1:200 solution of 40 per cent formaldehyde for twenty-one minutes.

The disease has been shown to result from seed infection by (a) planting naturally infected seeds in steam sterilized soil, (b) growing of diseased seedling from a naturally infected seed on a sterile plate of potato agar, (c) growing diseased plants on steam sterilized soil from seeds contaminated by contact with ground-up infected tissues.

Seedlings grown in an infected seed-bed carry the disease with them to the field.

Healthy seedlings transplanted to an infected field will become diseased. The period of weakness following transplanting appears to offer favorable conditions for the attack of the fungus.

Under storage conditions, the fungus passes up through the central axis and out through the leaves, causing them to loosen from the stem and become light in weight and papery. Pycnidia develop abundantly on exposed surfaces.

Artificial inoculation of a number of healthy heads gave the same results in storage as did the natural infection.

The most satisfactory seed treatment from the standpoint of seed germination was found to be immersion for twenty to twenty-five minutes in a 1:200 solution of 40 per cent formaldehyde, followed by washing in clean water.

Exposure of pycnidia from soft cortical tissues to a 1:200 solution of 40 per cent formaldehyde for twenty-one minutes was found sufficient to kill the contained spores.

Naked spores were killed in two to three minutes in a 1:200 solution of 40 per cent formaldehyde.

A treatment of twenty-one minutes with a 1:200 solution of 40 per cent formaldehyde was not sufficient to disinfect seeds which had been invaded by the fungus mycelium.

Spraying of the seed bed and seedlings with Bordeaux mixture, according to Manns' (8) recommendations, does not control the black-leg in the seed-bed under Wisconsin conditions.

Removal of diseased host tissues from badly infected soil by passing it through fine-meshed screens prevented infection of seedlings in this

soil. The check flats containing the diseased host tissues gave abundant infection.

Covering badly diseased host tissues with clean soil to a depth of four to eight inches prevented infection of seedlings in this soil.

Removal of diseased tissues from the soil and deep fall plowing are suggested as a means of control on already infested fields.

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BRIEFER ARTICLES

IMBEDDING AND STAINING OF DISEASED WOOD[1]

J. S. Boyce

The following methods are not presented with any claim to originality but are more or less well known practices modified for the study of certain lignified tissues invaded by fungus mycelium. Very few specific directions are to be found in the literature concerning this particular branch of microtechnique. General principles are sometimes proposed from which an investigator may, after much time and effort, evolve special practice for the problem under consideration.

DIFFERENTIAL STAINING

While studying the perennial mycelium of *Gymnosporangium blasdaleanum* (D. & H.) Kern in wood of *Libocedrus decurrens* Torrey (Incense Cedar)[2] it was found necessary to secure a sharper differentiation between host tissue and fungus mycelium, than that afforded by the untreated material. The following method has proven itself admirably adapted to this purpose.

It is unnecessary to employ any killing or fixing agent for the mycelium. The intra- and intercellular hyphae have been found perfectly preserved in small limbs, air dried for more than a year. The infected wood is first cut into blocks of suitable size for clamping directly into a sliding microtome. These blocks are then heated in a mixture of three parts of 95 per cent alcohol to three parts of distilled water and one part of glycerine. The mixture is kept just below the boiling point for from fifteen to twenty minutes in order to soften the wood.

After this softening process the blocks are sectioned into 95 per cent alcohol, sections 10μ to 15μ in thickness being easily obtainable. These sections are next stained for one hour in a mixture of nineteen parts of malachite green to one part of acid fuchsin, both being 1 per cent solutions

[1] The writer wishes to make grateful acknowledgment to Dr. L. I. Burlingame of Leland Stanford Jr. University for advice and assistance throughout the course of this work.

[2] Boyce, J. S. Perennial mycelium of Gymnosporangium blasdaleanum. Phytopathology **8**: 161–162 (April 1918).

in 30 per cent alcohol. The surplus stain is removed on filter paper, the sections are washed in 95 per cent alcohol for from five to ten minutes and then transferred to 70 per cent acid alcohol (70 per cent alcohol to which a few drops of concentrated hydrochloric acid have been added) until properly differentiated. This process is followed closely under the microscope. When the differentiation is satisfactory the sections are placed in absolute alcohol for about two minutes to completely dehydrate them and are then transferred to carbol-turpentine-cedar-oil clearer[3] for from five to ten minutes. From this clearer they are removed to xylol for ten minutes and then mounted in balsam under a cover glass or may be stored in xylol. Sections can be kept in this way for several weeks.

The advantage of the above method is primarily its rapidity. Sections can be mounted for study within two hours after the work is begun, but more precise differentiation can be obtained by staining overnight (approximately twelve hours) in malachite green, washing out the surplus stain with 95 per cent alcohol or drawing it with acid alcohol and then counterstaining from ten minutes to one hour with acid fuchsin. The progress of this counterstain should be watched under the microscope until the required intensity is obtained. In actual practice it is best to overstain and draw the surplus with acid alcohol until the desired differentiation is attained.

This method will undoubtedly prove applicable to other lignified tissues containing fungus mycelium, in some cases doubtless requiring a modification of the proportion of the two stains which may be accomplished by varying either the density of the solutions or the time of action.

IMBEDDING IN GELATINE

Some difficulty was encountered in securing sections of wood of Incense Cedar destroyed by dry-rot caused by *Polyporus amarus*.

This rot, as the name implies, is very dry and friable. Sections cannot be cut dry; they commonly crumble to powder under the knife. If the material is moistened sections may be obtained but they are exceedingly delicate, tearing easily and curling or folding badly when handled. To imbed in celloidin or paraffin is too long a process for quick work. Some substance therefore is required which combines speed with ease of handling, and at the same time keeps the imbedded material moist. Gelatine, originally used by Land[4] for imbedding admirably fulfills all of these re-

[3] Carbolic acid crystals liquefied, 1 part (weight).
Turpentine, 4 parts (volume).
Cedar oil, 2 parts (volume).

[4] Land, W. J. G. Imbedding in gelatine. Botanical Gazette **59**: 400 (May 1915).

quirements. Cubes of the decayed wood with faces 5 mm., or even less, square are most suitable because they section more readily and uniformly than larger sizes. These pieces are then heated or even boiled in a mixture of equal parts of 95 per cent alcohol and distilled water for from five to ten minutes in order to drive out all the air. As an added precaution they are then immersed in absolute alcohol for from ten to twenty minutes at the least. The material is next placed in liquefied gelatine about the consistency of molasses and then infiltrated for from one to several hours at a temperature of about 52°C. Granulated gelatine, readily obtainable at any grocery store, has been found better adapted to this purpose than sheet gelatine, since it dissolves more readily. The use of warm water greatly hastens the process. If the solution, when first made up, contains air bubbles, it should be placed in an imbedding oven until the bubbles all rise to the surface, when they can then be skimmed off. This may require several hours, depending of course on the density of the solution.

The cubes are next imbedded in paper trays which have been previously saturated with glycerine or melted paraffin. The gelatine is poured into the tray, and the cubes placed in the gelatine. They sink rapidly, and should be oriented so that the face to be cut is parallel with and separated from the bottom of the tray by a thin layer of gelatine. The trays are made deep enough so that there is a sufficient depth of gelatine to permit the resulting block, with its imbedded cube of wood, to be clamped directly into the sliding microtome. The gelatine is permitted to solidify, the paper imbedding tray is removed and the blocks are hardened in formalin for from twelve to twenty-four hours. They may then be kept for several weeks.

In sectioning, the trimmed gelatine block, clamped directly into the sliding microtome, is kept moistened with water. Sections 10μ to 15μ thick are easily obtained. These sections of decayed wood, each with its surrounding margin of gelatine, are transferred from the microtome knife to a solid watch glass filled with distilled water. Here they can be straightened out if necessary. The sections are now ready for mounting in glycerine or, which is preferable, in glycerine jelly. When the latter is used, the melted jelly is kept thinned by adding a little water. Glycerine jelly undiluted concentrates under the influence of heat before the sections can be properly arranged. The slide is placed on the warming stand and the required amount of thinned, liquefied jelly placed on the slide. The sections are then transferred by means of a section lifter to the jelly on the slide, suitably arranged and covered with a cover glass. Any surplus water will evaporate from the glycerine jelly during the process of hardening

If it is desired to stain the wood, bismarck brown in either aqueous or alcoholic solutions can be used. The sections are transferred from the

microtome knife directly to the stain. If an alcoholic solution is used the sections will curl badly but will immediately straighten out when they are placed in water. The sections should be stained in a 2 per cent solution for about one-half hour. The surplus stain is washed off in distilled water, then the sections are mounted as previously described. The decayed wood takes the stain strongly, retaining it, while the gelatine nearly regains its normal color after the washing process.

The results with differential staining were unsatisfactory. No stain was found which would act on the fungus mycelium in the decayed wood without at the same time deeply staining the gelatine.

IMBEDDING IN PARAFFIN

It was also found, that by the exercise of a little care the dry-rot could be imbedded in paraffin and sectioned on the rotary microtome. The decayed wood is treated as before described until it is placed in 100 per cent alcohol. Then it is left there for twelve hours. It is next placed for successive periods of twelve hours each in an imbedding oven at 54°C. in mixtures of 2 parts of 100 per cent alcohol to 1 part of cedar oil, equal parts of alcohol and cedar oil, 1 part of alcohol to 2 parts of cedar oil and finally pure cedar oil. Next, the cedar oil is gradually replaced by paraffin the process normally requiring about seven days until the material is in almost pure paraffin containing just the slightest trace of cedar oil. Care must be exercised however for if too much oil is left in the paraffin, sectioning will be an impossibility, the paraffin merely crumbling under the knife.

The material is imbedded in the usual way in the paraffin "flavored" as it were with cedar oil. The value of the cedar oil as an infiltrating agent is apparent in sectioning. The decayed wood is much softer, cutting more readily and easily than was found to be the case when other oils were used for this purpose.

Sections 5μ thick are easily obtainable on the rotary microtome but those of 15μ are more satisfactory, those below this thickness being too thin. The ribbons should be fastened to the slide by means of gum arabic and potassium bichromate solution, as proposed by Land.[5] This is superior to egg albumen for our purpose, since the sections are glued more firmly to the slide and there is no danger of ruining the preparation by overheating. The paraffin is dissolved away with xylol, and the latter washed off with 100 per cent alcohol. The sections can then be treated as desired, stained or tested with reagents.

[5] Land, W. J. G. A method of fixing paraffin ribbons to the slide with certainty. Botanical Gazette **59**: 398 (May 1915).

The imbedding and mounting process does not seem to affect the chemical properties of the wood. Free hand sections mounted in water showed no difference in behavior with regard to the commonly used reagents such as phloroglucin, anilin sulfate, chloro-iodide of zinc, etc., from wood treated as described.

The value of the method given is that the very delicate and easily disorganized sections are firmly attached to a slide and can be handled, tested and stained with ease whereas free hand sections of this dry-rot are difficult to work with. It is obvious, however, that this process should be resorted to only when free hand sections prove unsatisfactory.

Both the paraffin and gelatine methods are very probably applicable to other diseased woods of a similar type.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
SAN FRANCISCO, CALIFORNIA

A CONVENIENT HEATING AND STERILIZING OUTFIT FOR A FIELD LABORATORY

GEORGE L. PELTIER AND DAVID C. NEAL

During the summer of 1917, a more or less permanent field laboratory for the study of Citrus canker was installed in South Alabama. In fitting out the laboratory the list of equipment given by Reddick[1] was used as a basis. Due to the large amount of culture work involved in our investigations a more convenient and time saving heating apparatus was necessary than the blue flame kerosene stove recommended by Reddick. After looking over a number of outfits, the following apparatus was chosen, and with a few modifications, proved very successful.

The outfit includes an autoclave ("National Junior"), used in home canning work, consisting of a steel retort, base and burner (fig. 1) and a gasoline tank with four modified burners (fig. 2). The autoclave is heated by a gasoline-air mixture which produces an intensely blue flame, similar to that of a Bunsen burner. The size of the flame may be regulated at will, and fifteen pounds pressure may be obtained very quickly. The burners are attached by means of a hollow copper wire to a gasoline tank, which may be set in an out of the way place, and the burners located in any part of the laboratory. By means of valve connections, with the wire, and controls at the burners, the amount of the gasoline-air mixture can be regulated so that any size flame can be obtained. A tripod placed over the burners brings the vessels to be heated in close proximity to the flame.

[1] Reddick, D. Field laboratory equipment, Phytopathology, **2**: 172–174. A. 1912.

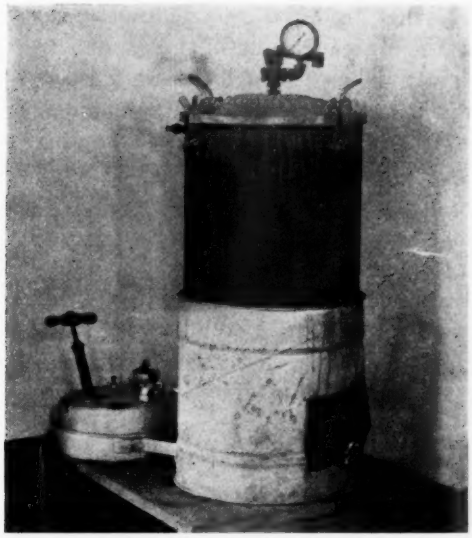

FIG. 1 "NATIONAL JUNIOR" AUTOCLAVE

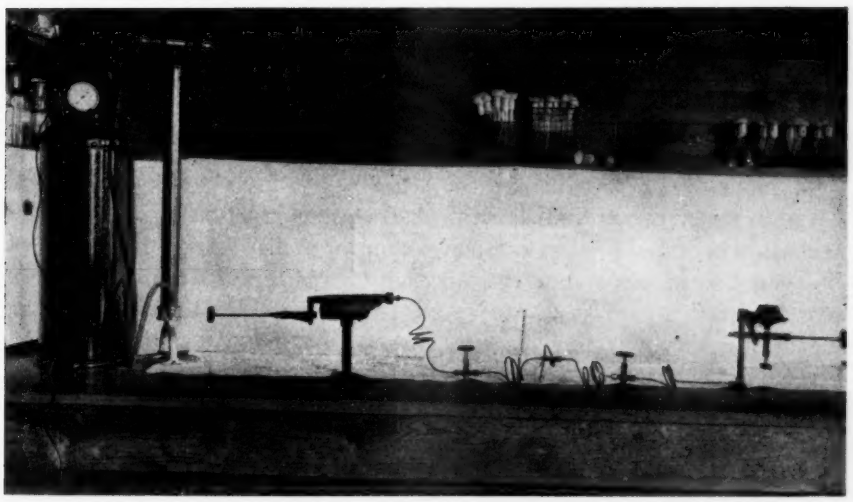

FIG. 2. GASOLINE AIR TANK, BURNERS, CONNECTIONS, AND ACCESSORIES

The burners give a flat blue flame. A modified burner was made to give a long flame similar to that produced by a Bunsen burner.

With this apparatus a great saving in time is effected and it is as convenient and successful as where gas facilities are at hand.

The total cost of the autoclave, tank, burners and accessories was only $65. It can all be packed in a box, and has a shipping weight of approximately 100 pounds. The whole outfit can be set up in a few hours in an open field, shed or building, and it is perfectly safe. One gallon of gasoline will run the autoclave continuously for ten hours, so that it is almost as economical as a kerosene stove.

Only two disadvantages have been encountered with the apparatus in the year that it has been in use. The burners must first be heated to a point high enough to cause the gasoline-air mixture to ignite. Wood alcohol is used for the purpose and from five to ten minutes are needed to ignite and regulate the burners. The gasoline-air mixture is regulated at the burner by means of a long needle valve, which is easily put out of adjustment if not carefully handled

As home canning has become popular, there are many kinds of steam pressure outfits and burners on the market, so that they are not difficult to obtain, and it may be that there are outfits on the market which will prove even more satisfactory than the one briefly described above.

ALABAMA EXPERIMENT STATION
AUBURN, ALABAMA

INCUBATION PERIOD OF CRONARTIUM RIBICOLA ON THE WHITE PINE

R. E. STONE

Within recent years a number of observations have been made on the incubation period of *Cronartium ribicola* on the White Pine. The different observers do not agree upon the duration of this period. Some observers have reported a two year life cycle while others have assigned other lengths of cycles up to five years.

In 1917, the writer found the aecial stage of this rust on pine trees that had been examined for three successive seasons without finding aecia. Originally there were plants of *Ribes cynosbati* among these infected pines. These Ribes plants were heavily infested in 1914. They were cut out in the spring of 1915 so that there was little chance of infection after that time. Altogether forty cases of infection were found and they were distributed as follows: Infection on 1913 wood 12, sporulated in May, 1917. Infection on 1914 wood, 28, sporulated in May, 1917.

In 70 per cent of cases found the period of incubation could not have been longer than two years and ten months, as infection could not have taken place earlier than July, 1914. It is also improbable that the incubation period was shorter than this, because the Ribes plants that were the source of infection had been removed in the spring of 1915 before the

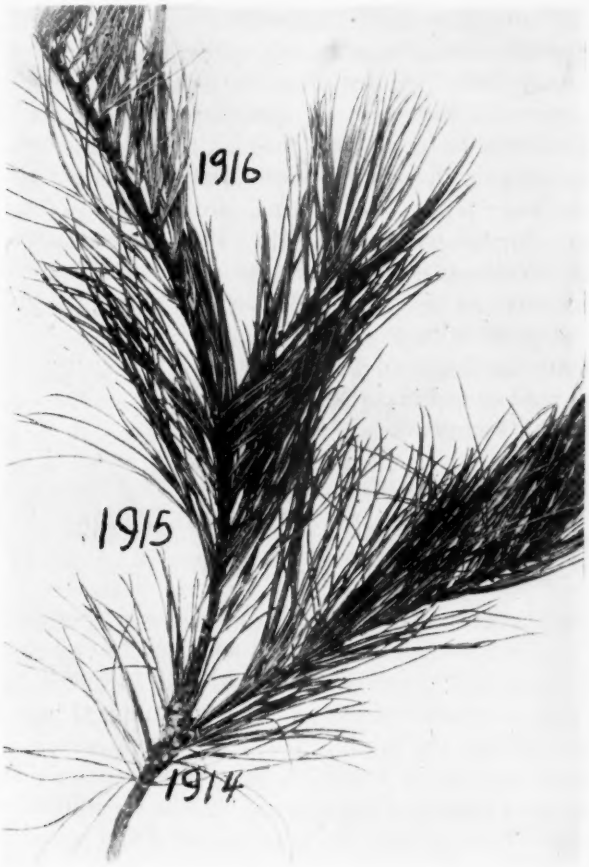

FIG. 1. THREE YEAR LIFE CYCLE OF CRONARTIUM RIBICOLA ON WHITE PINE

leaves had unfolded. This would indicate a three year cycle for the White Pine Blister Rust.

In 30 per cent of the cases infection had taken place on 1913 wood; the cycle is not so definitely shown. If infection took place in 1913 the rust here had a four year cycle and an incubation period of three years and ten months. The swellings showed no aecial scars and besides the

trees were examined three times in May and June, 1916, and no aecia found, so that 1917 was the first time of sporulation. It is possible that infection took place in 1914 on one year old wood and that the period of incubation was, as in the other 70 per cent of cases, a little short of three years. However, where the pustules occurred on 1913 wood it is probable that infection took place in 1913 and that here we have a four year cycle with an incubation period of three years and ten months.

Considering the specimens obtained in this case it is evident that the most common period of incubation is two years and nine or ten months with a life cycle of three years. Sometimes the incubation period is three years and nine or ten months with life cycle of four years.

The accompanying illustration shows a distinct three year life cycle. Infection took place late in 1914 and included the base of the terminal bud for that year. Sporulation began in May, 1917, before the season's growth had begun. In this case the incubation period was from August, 1914, to early May, 1917, or two years and nine months and the life cycle would have been complete in three years.

DEPARTMENT OF BOTANY
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A MORPHOLOGICAL AND CULTURAL NOTE ON THE ORGANISM CAUSING STEWART'S DISEASE OF SWEET CORN

LUCIA MCCULLOCH

During the past ten years this organism has been isolated by the writer fourteen times from diseased maize obtained from several states as well as from the District of Columbia. In each case, inoculations were made which resulted in typical infections and plates poured from the diseased maize plants yielded the organism which was inoculated. In none of these isolations has any true motility been observed, and no success has attended repeated efforts to demonstrate flagella by staining with various flagella stains. The organism has been observed and tested directly from the host as well as from various artificial media. Paired rods on the margin of a hanging drop often exhibit a rather vigorous tumbling motion but this must be regarded as Brownian movement, since cultures killed by heat show paired rods with the same amount and kind of movement.

As flagella do not occur on this organism the names *Pseudomonas Stewarti* and *Bacterium Stewarti* are inappropriate. It is suggested, therefore, that *Aplanobacter Stewarti* (Smith) is the proper name for this organism.

The cultural observation referred to is that in *Aplanobacter Stewarti* there are two distinct types of surface colonies as seen on peptonized beef

agar plates. One type has a smooth flat surface. The other has a definite central depression. At least half of the isolations made by the writer during the past ten years have been of the latter type. At first this depression is a small funnel-shaped crater; later the walls are practically perpendicular and as the floor of the opening is merely a thin film, the general appearance is that of a circular small window in a wide yellow frame. Colonies 7 mm. in diameter have windows 1 to 2 mm. in diameter. The crater appears early in the development of the colony and usually persists. Occasionally but not very often the crater fills in by normal growth. In old (ten to fifteen days) colonies often a secondary growth occurs as small colonies within the old one. These new areas of growth then obliterate the crater. In crowded plates (30 or more colonies to the square centimeter) this crater character is absent or so slight as to pass unnoticed. Always so far as observed all the colonies of an isolation are of one type or the other—not mixed. If one colony is smooth all will be, and vice versa. This crater character is not dependent on medium or temperature as proved by parallel poured plates of both types ofcolony.

The "cratered" strain when used for infecting corn remains cratered when reisolated from the diseased plants. Smooth surfaced colonies likewise reproduce true to type. Stock cultures kept for two years in the refrigerator still retain the original characters.

In virulence, character of infection, general cultural characteristics and morphology, including the lack of flagella, these two types appear to be identical. The only difference noted being in the behavior of the colonies on peptone-beef agar.

The appearance of the cratered colonies is shown on plate I.

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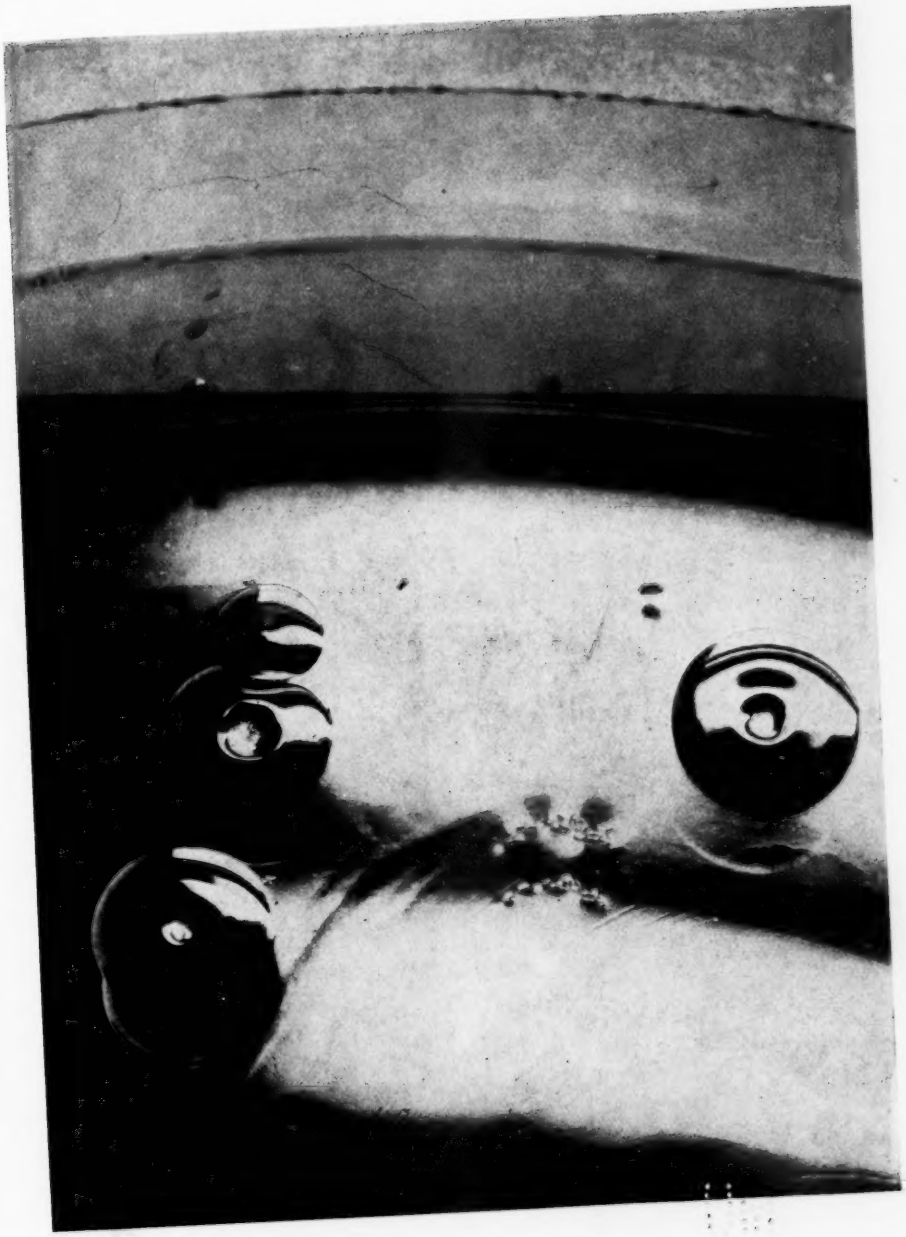
PLATE I

Cratered colonies of Aplanobacter Stewarti on + 14 peptone beef agar. The small broadly elliptical growths are buried colonies. The curves below the surface colonies are shadows. Organism isolated June 25, 1917, from Golden Bantam Sweet Corn. Photo. June 28, by very oblique light. × 9.



McCULLOCH: SWEET CORN DISEASE

REVIEWS

Citrus canker in Japan: a translation of the first description of this disease, from the Japanese.[1] "A new kind of Pathogenic Microbe: being No. 8 of the organisms injurious to citrus." From "Nippon no Mikan," by Kumanosuke Abe, Pub. in M. 37, v. (May 1904), p. 162–165.

This organism was first discovered in Meiji 32 (1899) in the nursery of Tanushimaru on Washington navel orange. At that time about 70,000 to 80,000 navel orange trees had been planted in this same district and about 70 to 80 per cent of these trees were found affected by this disease.

The first symptoms that appear are on the under surface of the leaves and are small rusty colored spots a little elevated from the surface. This spot is easily transmitted to the other young foliage developed in the same year; also attacks the shoots causing the destruction of this part of the tree.

It seems to attack only summer shoots and never attacks the spring shoots as far as the writer has observed. This fact leads the writer to think that the organism does not develop until the summer shoot comes out; but at the time of the growth of the summer shoot it makes a vigorous growth, attacking the leaves and twigs while they are still soft, readily spreading from one tree to others.

Whenever the disease occurs on the young tree it never causes the plant to die; but as it destroys the summer shoots entirely it disturbs a great deal the healthy development of the plant so that it causes a big loss to the nurserymen.

In the nursery of Tanushimaru there are many plantations of Satsuma trees which, in many cases, surround the navel nurseries; but no infections were discovered on the Satsuma orange even where navels were attacked rather severely by the disease.

The other varieties like Natsu-daidai, Yamabuki-Mikan and the Trifoliata orange, all of them considered to have similar habits to the navel orange, were found to be affected slightly by the disease, and the suscepti-

[1] While no name is given by the author to the organism or to the disease itself, a study of the symptoms described leaves no doubt that the writer was dealing with true citrus canker, caused by *Pseudomonas citri*, Hasse. Though Mr. Abe's description was not published until May, 1904, his discovery of the disease dates from 1899, and the manuscript was prepared for publication probably in 1900 or 1901, as it is mentioned by Viscount Kano in his preface dated February 8, 1901.

Note by Tyozaburo Tanaka Translator, U. S. Department of Agriculture.

bility of the navel orange is also considered due to the softness of the twigs, leaves and bark.

In the present stage (as far as the writer has observed) it is rather difficult to decide whether or not this is a special disease of the navel orange; but in its general aspects it seems to be quite possible that it is.

The disease may have been in existence in other nursery districts without being developed on account of the unfavorable climatic and soil conditions. It was formerly the opinion of the writer that the conditions at Tanushimaru just happened to render favorable the development of the disease.

The writer (i.e., Mr. Abe) had occasion to ask Prof. Tamari about the cause of the disease, and the nature of the organism, but his reply was that it had never been found in this country.

The writer made a careful search in Wakayama-ken from which nearly all navel orange trees of Tanushimaru originated and did not discover any affection of this character on the plants there, notwithstanding he fully expected to find such affection. This fact will possibly prove the above hypothesis of the writer. Owing to the fact that the nature of the organism has not yet been thoroughly studied, no attempt is made in this paper to give either the name of the disease or the organism.

The propagation of Citrus has been an industry for a long period in Tanushimaru, but people there say they never noticed before such a disease as the writer here describes. It is quite possible that the organism will attack not only the nursery stocks, but the fruits, though this has not, however, been observed as yet.

The severity of the infection is more pronounced in the wet years, and spreads more rapidly at such times.

As to the methods of prevention and extermination, it has not been sufficiently experimented with as yet; but it would be wise to take the precaution to burn up all diseased leaves and twigs which should be cut off and collected immediately upon discovery of the affection.

The application of Bordeaux mixture once in the springtime, or, preferably once more during the winter season is recommended for the prevention of the disease.

TYOZABURO TANAKA

Annals of the Phytopathological Society of Japan. In beginning the publication of a journal devoted exclusively to Phytopathology our Japanese colleagues have taken an important step which will be welcomed the world over. We may predict for the *Annals* the same usefulness that is served by our own journal, *Phytopathology*, of which it is an exact duplicate in size, appearance and general makeup.

The *Annals* is issued by the society under the presidency of Prof. M. Shirai; a committee of nineteen members of the society; and under the managership of N. Suemastu, T. Hemmi, and U. Bokura. The frequency of issue and price per volume is not mentioned but can be obtained on application to U. Bokura, Imperial Agricultural Experiment Station, Nishigahara, Tokyo, Japan.

The first number contains the following articles: On the development of plant pathology in Japan: A brief historical sketch, by Dr. M. Shirai (English with occasional notations in Japanese); A preliminary report on a late-blight resistant strain of potato, by S. Ito (in English with summary in Japanese); Vorläufige Mitteilung über eine neue Anthraknose von Euonymus japonica, by Kakewo Hemmi (German with summary in Japanese); On the grain of barley or wheat, infected by smut fungus through the flower, by Shikutaro Miura (Japanese with brief summary in English); Soy bean cake as a substitute for peptone in the preparation of the nutrient media, by S. Hori and U. Bokura (English and Japanese); thirty pages of reviews of pathological literature and notes.

The launching of such a periodical at the present time is evidence of the seriousness of purpose of our sister society and, bearing in mind our own trepidation and early financial stress, merits our prompt support.

D. REDDICK

PHYTOPATHOLOGICAL NOTES

Bean pod meal for culture media. In many institutions an agar medium made from an infusion of fresh bean pods is used to a considerable extent in phytopathological work. The greatest drawback to a more extended use of this medium has been the difficulty of obtaining fresh beans at all seasons of the year. On account of this, it has been the usual practice either to make up a considerable amount of agar at the time beans are in season or else can the beans and then obtain the infusion from the canned product. Neither of these practices is entirely satisfactory. Bean agar that is kept for some time turns darker in color and dries out to a considerable extent and agar made from the canned product is usually darker in color and furthermore is quite variable in composition.

A simpler and more satisfactory method of having bean agar at any season of the year is by the use of bean pod meal. The beans are picked in season, cut up into small pieces and dried to crispness with artificial heat. Drying the beans for a short time in a hot air sterilizer and then transferring them to an ordinary paraffin oven has given us very good results. The heat in this oven is not high enough to darken the beans and is sufficient to dry them thoroughly in a day or so. After drying, the material is

ground into a fine meal and put away in bottles for future use. We have not kept any of this meal for any considerable time, but there seems to be no reason why it should deteriorate or turn darker in color especially if it is kept in a dark place.

In making an agar medium about 20 grams of the meal is used for each liter. As beans dry down to a little less than one-tenth of their original weight, this corresponds to about 250 grams of the green beans, the amount generally used in making bean pod agar. In making the meal agar, a half hour's infusion at 50° to 60°C. is sufficient. After filtering the infusion, the agar is made in the ordinary manner.

Agar made from bean pod meal is slightly, if any, darker in color than agar made from the fresh pods. Careful tests with a number of organisms, both fungous and bacterial, have shown an identical growth on the agars from the meal and from the fresh bean pods.

The use of bean pod meal not only simplifies the preparation of the agar medium, making it available at all seasons of the year, but also furnishes a more constant medium. Two batches of agar made from a well mixed bottle of the meal are much more uniform than two batches made from canned beans or even from fresh pods.

C. W. EDGERTON

A method for making permanent mounts of entire colonies of some fungi in plate cultures. In investigating relationships among strains of fungi, it is often desirable to preserve entire fungus colonies for record purposes. It is unsatisfactory to keep agar plate cultures permanently whether mounted on glass or on sheets of celluloid. Since the chief difficulties seem to be due to the agar medium an effort was made to wash out the medium from dry plate cultures of *Rhizoctonia solani* Kuhn and *R. microsclerotia* Matz with satisfactory results. The following procedure was employed: Single sclerotia were planted in the center of sterilized petri dishes containing a small amount of clear corn meal agar, sufficient to insure a growth covering the entire inner surface of the bottom dish. After the colonies had developed and the agar medium had been allowed to dry out completely at room temperature, the plates were placed in boiling water until the agar was entirely dissolved. This was followed by rinsing in clean water and dehydrating with alcohol. The fungus colonies, which during the period of growth had reached and grown upon the vertical walls of the bottom dish, adhered to the glass and were plainly visible. To emphasize distinctness the colonies were stained with saffranin and gentian violet, the excess stain was removed with alcohol, and they were allowed to dry. With fungi which produce copious growth it may be best to leave them unstained, as the stain will tend to obscure rather than to define. The

use of saffranin alone, removing the excess stain with water, is equally satisfactory.

The principal points involved in the above method are:

1. The fungus colony should be allowed to cover the entire surface of the culture dish, and if possible induce a marginal growth on the free-from-medium surface of the vertical walls of the dish. This will tend to hold the colony intact during the process of washing.

2. A minimum amount of agar medium should be used in the plates intended for mounting.

3. The agar film must be thoroughly dry and adhering to the dish.

4. A complete removal of the agar is essential if staining is desired, as the agar will absorb the stain.

J. MATZ

A new method for "hanging drop" cultures. The usual practice is followed in the preparation of the chamber, the clean cover glass to carry the culture is flamed and held between the useful Cornet forceps. Meanwhile, if a solid medium is used (agar or gelatine preparation), a tube is melted in the water bath. Then a small cover glass or a small piece of cover glass is cleaned and sterilized by flaming, dipped into the molten medium, and transferred to the cover glass held in the forceps. Enough medium is transferred in this manner to observe the minutest organism in a very agreeable manner. The film is even in thickness, and after a little practice there should be no air bubbles in the contact film. The whole culture is kept moist very satisfactorily after moistening the chamber and sealing with vaseline. When incubation at higher temperatures becomes necessary, the cultures may be placed in a stender dish the walls of which are covered with moist filter paper. Inoculation may be made on either side of the film. This method possesses promising possibilities for the study of anaerobic organisms.

H. T. GÜSSOW

Microphotography simplified. It is presumed that investigators are familiar with the Bausch and Lomb Simple Drawing Apparatus, the usefulness of which lies in its simple construction and in the reflecting mirror clamped to the eyepiece tube of almost any standard microscope. The arrangement provides for a 50 to 30 diameter magnification, depending upon objective, eyepiece and distance of paper from mirror. When using such apparatus a substitution of the ordinary drawing paper by some photographic paper naturally suggested itself. A preliminary test during which all superfluous light was eliminated by covering the microscope and drawing apparatus with a black cloth proved quite satisfactory, though of course

negative prints resulted. These will answer in many instances, as for purposes of record. It is not necessary to further explain the rapidity with which accurate copies of any microscopical preparation might be taken by these means. The next step was the use of a photographic plate instead of paper. For this purpose a square wooden box was constructed, the dimensions of which were according to size of plate which it was desired to use. The box is to be left open at the top. The bottom is provided with grooved guiding strips of wood similar to the back of the camera to secure an ordinary dark slide in its position. We used a plate carrier for a 5:7 plate and a slit was made at one of the sides through which the dark slide could be pushed in along the guides. A black light-tight cloth covered the box and was fastened around the microscope to shut out all rays of light with the exception of those passing through the microscope. A 90° hand-feed arc lamp is used as an illuminator with bull's eye condensor. We found this most satisfactory and the preparation of the box which was painted inside with India ink, required little more time than is generally necessary to set up one's microphotographic cameras. The box is then pinned in position to the bottom of the drawing apparatus. To focus the object, place an unexposed photographic plate film side up into the dark slide, turn on and adjust the light and focus the image. When satisfactorily adjusted, turn off the light and replace the plate used for focusing by another unexposed one. Insert the slide into box, place a piece of white paper on it, turn on the light, adjust its brilliance, then remove the paper and securely cover the box with black cloth. Shut off rays by inserting and holding piece of cardboard between source of light and object, draw open the slide and make the exposure. The use of a Zettnow filter is recommended for stained sections. The method is simple, accurate and saves lots of time, and the results are excellent for general purposes as well as for making lantern slide negatives. Difficulties are experienced in photographing bacteria, but these we hope will yet be overcome.

H. T. GÜSSOW

Personals. Mr. Walter S. Beach, who has just taken his docotorate with his thesis in plant pathology, at the University of Illinois, has been appointed as instructor for plant pathological research in the Pennsylvania State College. He will have charge of a separate laboratory located near Philadelphia, and is to take up his work at once.

Mr. Julius Matz, formerly assistant in plant pathology at the Florida Agricultural Experiment Station, has recently accepted a position as agent in the Bureau of Plant Industry, with headquarters at Loxley, Alabama.

Mr. E. J. Hemmer, recently instructor in agriculture and botany and principal of the Montpelier, Indiana, high school, has been appointed assist-

ant pathologist in the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, and will take up pathological extension work in Indiana, with headquarters at Purdue University.

Dr. Chas. H. Otis, Professor of Botany, Department of Biology, Western Reserve University, Cleveland, Ohio; Dr. J. C. Gilman, Professor of Biology, Ripon College, Ripon, Wisconsin, and formerly of University of Wisconsin; and Prof. H. S. Stahl, Professor of Biology in Virginia Polytechnic Institute, Blacksburg, Virginia, have accepted summer employment with the Plant Disease Survey commencing June 1. They will be engaged in making a survey for various root rots of corn, particularly those caused by Fusarium spp. This work is conducted in coöperation with the Office of Cereal Investigations, with Dr. G. N. Hoffer, agent for that office, directing the investigational part of the work.

APPENDIX

LA FAYETTE CONFERENCE

MINUTES OF THE WAR EMERGENCY CONFERENCE OF PLANT PATHOLOGISTS OF THE NORTH CENTRAL EAST

At the meeting of plant pathologists held at Purdue University, La Fayette, Indiana, March 15–16, 1918, representatives were present from the various states as follows: Colorado, W. G. Sackett; Illinois, H. W. Anderson, W. S. Beach, Geo. H. Dungan, L. E. Miles, Ray Nelson, F. L. Stevens; Indiana, J. C. Arthur, John W. Barringer, S. Conner, Stanley Coulter, J. B. Demaree, G. H. Gillespie, L. Greene, L. F. Heimlich, G. N. Hoffer, Richard M. Holman, H. S. Jackson, C. A. Ludwig, E. B. Mains, R. R. Mellett, Geo. A. Osner, E. W. Phelps, E. J. Petry, F. J. Pipal, Orda R. Plunkett, Burr Prentice, Harry Reed, F. E. Robbins, H. R. Rosen, J. H. Skinner, Geo. Hum. Smith, E. H. Toole, P. A. Tetrault, J. M. Van Hook, Grace Wineland, C. G. Woodbury, W. H. Wright; Michigan, E. W. Brandee, G. H. Coons; Minnesota, E. C. Stakman; New York, H. H. Whetzel; Ohio, A. D. Selby, C. R. Stevenson; Pennsylvania, Frank D. Kern; Washington, D. C., Chas. Brooks, W. W. Gilbert, H. B. Humphrey, G. R. Lyman; Wisconsin, L. R. Jones, R. E. Vaughan.

After a brief résumé of the work of the War Emergency Board the important crop diseases of the region were discussed and leaders elected as follows:

Cereals diseases

1. Corn diseases. G. N. Hoffer, Leader. Prof. Hoffer reported that three strains of Fusarium may damage corn, chiefly affecting the ear, but also the root leading to barren stalks, or nubbins. Aphis injury favors the entrance. Resistant strains of corn have maintained their quality during six years trial. Resistance may be determined by germination tests. Early maturing varieties are disease escaping.

Damage caused by corn rust and smut was also discussed. It was stated that smut may have caused a 3 per cent loss last year, that smutted stalks mature less hard corn; sweet corn smuts worst. While sanitation is the most reliable control method, its practical value is yet to be finally demonstrated.

Dr. F. L. Stevens was chosen leader of a special corn smut eradication project. He urged securing coöperation of boy scouts to cut out smut boils on a large scale and suggested the possibility of using young smut boils as food. Stakman suggested securing services of farm boy cavaliers.

2. Wheat scab. H. S. Jackson, Leader. Scab was reported as being one of the most serious diseases in Illinois, Indiana and Ohio, and it occurs widely from Minnesota to the Atlantic Coast. Control is not well understood but it was recommended to clean, grade and disinfect seed. Hoffer stated that the Fusarium causing wheat scab closely resembled that producing a corn root disease. Wheat following corn has more scab than when following other cultivated crops. Professor Jones reported investigations under way in Wisconsin on the relations of Fusarium to occurrence of black point or ebony tip, including study of soil temperature relations to seedling injury. Stakman reported studies on Fusarium saprogenesis and relation of soil temperature with spring wheat. It is found on other cereals, emmer and some grasses. Cooperative field tests, with treated vs. untreated seed will be run by Stakman, Coons, Dungan, Jones, Jackson, Selby and Gregory.

3. Grain rusts. E. C. Stakman, Leader. Rust work was reported, including epiphytology, overwintering, biologic specialization, and relation of temperature with *P. cornata*, *P. dispersa*, *P. simplex*, as well as *P. graminis*. Barberry eradication as a controlling factor in rust epidemics is being pushed in N. Mississippi Valley States. State leaders are Freeman, Minnesota; A. G. Johnson, Wisconsin; Coons, Michigan; Stevens, Illinois; Jackson, Indiana.

L. R. Jones recommended that the data compiled by Stakman for a Farmers' Bulletin on grain rust be mimeographed at once and sent to all experiment station pathologists and those concerned with barberry eradication.

4. Barley and rye diseases. A. G. Johnson, Leader. Dr. Johnson was urged to continue his studies on seed treatment and Helminthosporium.

5. Oat diseases. G. N. Hoffer, Leader. Sterility of oats due to Fusarium infection is to be studied by Hoffer; sterility due to physiological troubles by Holman; and studies on sterility due to bacterial trouble were assigned to Miss Elliott, of Wisconsin.

6. Grain seed treatment. A. G. Johnson, Leader. This project was considered as one of the most important. The widest possible coöperation and assistance in securing reliable data on smut losses is needed. Lyman was directed to furnish all interested with uniform blanks, now being prepared by Lutman for the eastern section. Studies on effect of treatment on germination should be continued several seasons; $\frac{1}{40}$ acre plots were suggested as the unit in determining yields.

Truck crop diseases

1. Potato diseases. G. H. Coons, Leader. The more important diseases were listed as: late blight, early blight, scab, Fusarium wilt, Rhizoctonia, black leg, mosaic. Stakman commended the seed plot method as succeeding in Minnesota. Whetzel urged the value of field inspection as compared with the more exacting seed certification based on New York experience. Coons advised meeting emergency needs for increased potato seed treatment by reducing time of soaking to fifteen minutes with formaldehyde and one-half hour with mercuric chloride. Jones urged the importance of diligent scouting for the first appearance of late blight. Coons stated that the midsummer weather rather than later, determines whether late blight is to be epidemic.

Recommendations for potato seed treatment and potato spraying were drawn up by a committee consisting of G. H. Coons, L. R. Jones and W. W. Gilbert. In these recommendations both corrosive sublimate and formaldehyde were advised for treatment with preference for the former where Rhizoctonia is at all serious. The shorter treatments as recommended by Coons were believed to be worthy of consideration.

2. Bean diseases. M. F. Barrus, Leader. Anthracnose and bacterial blight should receive most consideration.

3. Tomato diseases. F. J. Pritchard, Leader. Fusarium wilt and Septoria blight should receive most attention.

4. Cabbage diseases. L. R. Jones, Leader. Yellows and club root are to receive special consideration, in coöperation with C. R. Orton of the eastern section.

5. Onion diseases. J. C. Walker, Leader. Neck rot, anthracnose, and smut should receive special consideration. Coöperation with Whetzel and Anderson of eastern section was advised.

Fruit diseases

H. W. Anderson and Chas. Brooks, Leaders. The discussion showed need for further work on comparative effects of dusting vs. spraying.

Resolutions were adopted emphasizing the importance of fruits as foods and the effect of fruit diseases on the food supply, and urging adequate support for the prosecution of fruit disease work. A resolution was also passed requesting that much needed data collected by the Fungicide and Insecticide Board on the value of commercial spray materials be made available, as quickly as possible.

Transportation and storage diseases

Neil Stevens and G. K. K. Link, Leaders. Coöperation will be maintained with inspectors from the Office of Markets and a special inspector, which the Michigan Experiment Station proposes to retain in Chicago. Growers and shippers are to receive prompt reports on losses and remedial measures. The Bureau of Plant Industry will provide the inspectors with an up-to-date manual of diseases likely to be found.

Culture and specimen exchange

F. L. Stevens was appointed leader to inaugurate a system for the readier exchange of plant pathological specimens.

G. R. Lyman was urged to inaugurate at once a pure culture bureau in connection with his office.

Fungicides and insecticides

G. H. Coons reported that wholesale supplies seemed adequate for the work ahead. Heavy buying of spray machinery is reported by dealers and manufacturers.

Extension work

Extension methods used in New York, Michigan, Indiana and Wisconsin were outlined by pathologists from these states. Sample leaflets were exhibited and the

method of presenting extension information was discussed. It was felt that the use of posters had been overdone and that personal contact through meetings, schools, visits and correspondence was the most promising line of attack.

Man power

The man power situation was looked upon as critical and it was agreed that every teacher of botany and plant pathology should train his men as thoroughly as possible along the lines in which they can be of the most service.

The following resolution was adopted:

"WHEREAS there is urgent need of trained plant pathologists in food production service,

Resolved by the LaFayette conference of Plant Pathologists, that L. R. Jones, F. D. Kern, and two others to be selected from the western territory, be authorized to act for all American plant pathologists as a channel for handling individual cases for deferred military classification, or recalling men already in service."

It was suggested that this resolution be presented to the other conferences to be held in Nebraska and California, and taken up by letter with the eastern and southern conferences.

Plant disease survey

A resolution was adopted setting forth the value of an adequate plant disease survey and urging the development of the Federal office that has to do with this type of work.

R. E. VAUGHAN,
Secretary
G. H. COONS,
Chairman

LITERATURE ON PLANT DISEASES.[1]

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

May, 1912

American Phytopathological Society. Report of the ninth annual meeting, Pittsburgh, Dec. 28, 1917 to Jan. 1, 1918. Phytopathology **8**: 178–190. Ap. 1918. Including report of the chief editor of Phytopathology.

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[1]This list includes references, both American and foreign, to the literature of plant pathology and mycology of interest to pathologists. Foreign references published since January 1, 1917, have been included beginning with the list appearing in v. 7, no. 3, June, 1917.

All authors are urged to coöperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles published outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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